ORIGINAL RESEARCH

Evaluation of Fluoride Uptake of Two Fluoride Varnishes into and onto the Enamel Surface at Different Temperatures: An *In Vitro* Study

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Abstract

Background: Fluoride (F) is an effective anticaries agent and can be delivered through various mediums at different concentrations. The ability to increase the enamel resistance to acid by decreasing solubility through the incorporation of F into the enamel apatite structure is the primary function of these agents. The efficacy of topical F can be determined by measuring the amount of F incorporated in and on human enamel.

Aim: To compare the F uptake into and onto the enamel surface of two different F varnishes at different temperatures.

Materials and methods: In this study, 96 teeth were divided equally and randomly ($n = 48$) into two experimental groups (group I and group II). Each group was further divided into four equal subgroups ($n = 12$); depending on the temperature (25°, 37°, 50°, and 60°C) they were subjected to Fluor-Protector® 0.7% and Embrace® 5% F varnishes were allocated to experimental groups I and II, respectively, and every sample was individually treated with the assigned F varnish. After the varnish application, two specimens from each subgroup, the group I and group II ($n = 16$), were mounted for hard tissue microtome sectioning for scanning electron microscope (SEM) analysis. The remaining 80 teeth underwent potassium hydroxide (KOH) soluble and KOH-insoluble F estimation.

Results: Group I and group II, both, showed maximum F uptake of 2817.07 ppm and 1626.8 ppm at 37°C temperature and the lowest of 1168.9 ppm and 1068.93 ppm at 50°C, respectively. The intergroup comparison was carried out using an unpaired *t*-test and the intragroup comparison was done using one-way analysis of variance (ANOVA) univariate analysis.

Post hoc Tukey test was performed for pairwise comparison between different temperature groups. In group I (Fluor-Protector®), the difference in F uptake was statistically significant when the temperature was increased from 25° to 37° C (mean difference = −9.90, $p = 0.016$). In group II (Embrace®), a statistically significant difference in F uptake was observed when the temperature was increased from 25° to 50°C (mean difference = 10.00, $p = 0.003$) and 25° to 60°C (mean difference = 13.38, $p = 0.001$), respectively.

Conclusion: Fluor-Protector® varnish proved to have better F uptake in comparison to Embrace® varnish on human enamel. Topical F varnishes were most effective at 37°C, which is close to the standard human body temperature. Thus, the application of warm F varnish ensures more uptake of F in and onto the enamel surface for greater protection against dental caries.

Keywords: Enamel surface remineralization, Fluoride uptake, Fluoride varnish, Potassium hydroxide insoluble fluoride, Potassium hydroxide soluble fluoride, Temperature.

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Introduction

Dental caries, an infectious microbial disease of multifactorial origin, is the result of tooth demineralization due to the interaction of diet, host factors and microbial flora over a period of time, leading to the destruction of calcified tissues of the teeth.[1] There are many preventive strategies that have been advocated to counter dental caries, and among them, F application is a widely accepted and extremely effective treatment modality.[2] American Dental Association has also recommended periodic topical F treatment for children and adults who are at moderate to high risk of developing dental caries.[3]

The kinetic effect of F is mainly appreciated by two methods. The first is the incorporation of KOH-insoluble F compounds into the enamel. The alkali insoluble F (F bound to the apatite) is KOH insoluble F, which is structurally incorporated F in the enamel crystallites and prevents demineralization of the enamel during an episode of acid attack.[4] Alternatively, calcium-F (CaF_2) like precipitates, composed of KOH-soluble F, are formed and deposited on the enamel surface. Studies have shown that these deposits are highly soluble and release large amounts of F during a drop in the pH; hence, it provides a significant cariostatic effect and promotes remineralization on the enamel surface.[5]

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An F dental varnish can be defined as a lacquer or liquid made from a natural or synthetic base in which F salts are dissolved in a solvent, such as ethanol.[6] The development of F varnish began in the 1960s following the observations of Mellberg, who demonstrated large amounts of F release from enamel within the first 24 hours of topical application of acidulated phosphate F preparations.[7] Although different vehicles can be used for the application of F; varnish has been the most efficient, effective and widely used mode of topical F application. It has certain advantages over other methods, such as minimum time required for application, high F-uptake, and biannual application with minimum discomfort and maximum patient acceptability.

It is well established that F uptake in the enamel can be increased with an increase in F concentration. Recent studies have also shown that a mere increase in the temperature of F solution results in a considerable increase of F incorporation into and onto the enamel surface.[8,9] However, the researchers have only examined the effect of temperature on F gels and solutions. There is no published literature that investigates the effectiveness of F varnish at warmer temperatures.

Hence, the study was designed to investigate the effect of topical F varnishes at increased temperatures on the formation of both KOH-soluble and KOH -insoluble F on the enamel surface.

Materials and Methods

The present *in vitro* study was a parallel randomized controlled trial designed to determine and compare the F uptake in and onto the enamel surface of two different F varnishes at different temperatures.

A well-defined protocol for the intended study was submitted to the Institutional Ethics Review Board, and ethical clearance was obtained before the commencement of the study. The study was conducted in the Department of Pedodontics and Preventive Dentistry over a period of 3 months from May to July 2019.

Sampling Technique

A simple random sampling method was employed to allocate tooth samples into two different test groups.

Inclusion Criteria

- Freshly extracted human maxillary or mandibular premolars.
- Teeth extracted for orthodontic reasons.
- Complete intact tooth.

Exclusion Criteria

- Teeth with visible cracks under a microscope.
- Teeth with caries and white spot lesions.
- Teeth with a congenital anomaly.
- Teeth with preventive or restorative treatment.
- Teeth with stains and fluorosis.

Sample Size Estimation

A sample size of 96 teeth (n = 48 per group) was determined by considering the standard deviation of a previous study,[4] using the hypothesis testing for two means (equal variances).

Preparation of Specimens

First Step

Freshly extracted premolars (n = 100) were obtained from the Department of Oral Surgery and Pedodontics of a dental institute. The teeth were cleaned in pumice-water slurry and 0.3 µm alumina paste, with a polishing brush using a handpiece at low speed (5,000 rpm) to remove any debris or calculus deposited on the tooth surface.[7] The samples were then preserved in an atmosphere of 100% humidity at 4°C until use.

The preserved teeth were further examined under a microscope, and a final total of 96 teeth which met the inclusion/exclusion criteria, were selected for the study. The roots of individual teeth were then severed, 2 mm coronal, to the cementoenamel junction with the help of a water-cooled diamond disk. The prepared teeth were then embedded in self-polymerizing resin (dental pulp injury) in a Teflon mold with an exposed buccal enamel surface (6 × 7 mm). The embedded blocks of enamel were removed from the mold and stored at 100% relative humidity.

The enamel surfaces were later polished using fine grit sandpaper (CH2-HX#1000, Noritake Coated Abrasive), followed by thorough rinsing of the freshly exposed surface in ultrapure water using an ultrasonic cleaning device.

Allocation of Enamel Blocks

Then 96 enamel blocks were divided equally and randomly (n = 48) into two experimental groups (group I and group II). Each group was further divided into four equal subgroups (n = 12), depending on the temperature (25°, 37°, 50°, and 60°C) they were subjected to.

Selection of F Varnish

Two commercially available F varnishes with different concentrations were selected for the present study.

- Fluor-Protector® 0.7% (IvoclarVivadent® AG FL - 9494 Schaan Liechtenstein).
- Embrace® 5% (Pulpdent® Corporation, MA 02472 USA).

Fluor-Protector® 0.7% and Embrace® 5% F varnishes were assigned to experimental groups I and II, respectively.

F Treatment

Prior to the F application, every specimen was thoroughly rinsed with ultrapure water and blotted dry. The varnish was dispensed from the pouch (Embrace® varnish) and bottle (Fluor-Protector®) into two separate autoclavable plastic dispensers, respectively. These plastic dispensers were placed in a thermostatically controlled chamber to obtain a temperature of 25° ± 1°C. This was followed by the application of a thin layer of varnish onto the exposed enamel surface of the specimens (n = 12), with the help of a brush, in an aseptic condition. The above-mentioned steps were repeated at different temperatures of 37°, 50°, and 60°C for each of the two experimental groups. The pH of varnishes was measured using pH paper.

Thereafter, two specimens from each of the four subgroups (n = 16), from groups I and II, were remounted cylindrically and subjected to hard tissue microtome sectioning for SEM analysis. The remaining 80 samples were processed for KOH-soluble and KOH-insoluble F estimation, respectively.

Assay of KOH-soluble F

The required solution of 1 M KOH was prepared by adding 56.11 gm of KOH in 1 L of distilled water. A total of 80 separate plastic bottles were procured for each sample of both test groups. 10 mL of 1 M KOH was dispensed in each of the 80 plastic bottles and were labeled appropriately for group I (n = 40) and group II (n = 40) at four different temperatures. The F-treated specimen was rinsed

for 1 minute in a stream of ultrapure water and immersed in the assigned plastic bottle. The plastic bottles were then kept for 24 hours on an agitator for gentle agitation (100 rpm) at room temperature to remove loosely bound F from the tooth surface.[10]

To carryout the estimation of F concentrations, it was imperative to formulate the total ionic strength adjustment buffer (TISAB II) solution and calibrate an F-ion-selective electrode. TISAB II was prepared by adding 500 mL of distilled water in a beaker of 1 L capacity. The distilled water was then infused with 57 mL of glacial acetic acid and 58 gm of reagent-grade sodium chloride, and the beaker was placed in a water bath for cooling. 5 M Sodium hydroxide (NaOH) was carefully added into the solution until the desired pH between 5.0 and 5.5 was recorded, using a calibrated pH electrode. The solution was then cooled to room temperature and transferred to a volumetric flask. The TISAB II solution was finally produced by diluting the prepared solution with distilled water up to the 1 L mark of the flask.

The F-ion-selective electrode was calibrated with a different standard concentration of F solutions. 2.21 gm of sodium F was first dissolved in 100 mL of distilled water to obtain an F concentration of 10,000 ppm. Thereafter, 10 mL of the fluoridated 100 mL solution was added to another 100 mL of distilled water to attain a concentration of 1,000 ppm F. This step was further repeated to achieve concentrations of 100, 10, and 0.1 ppm to calibrate the ion-selective electrode for 0.1–10,000 ppm of F concentrations with an error of 5%.

To calculate the KOH-soluble F, each tooth sample was first immersed in a KOH solution for 24 hours. Around 2.5 mL of this KOH solution (aliquot) was extracted, to which 2.5 mL TISAB II buffer was added to make the final solution of 5 mL, for each specimen. F ion concentration of the 5 mL solution was measured using an F-ion-selective electrode (Thermo Scientific, Waltham, Massachusetts, United States of America), and the KOH-soluble F was quantified as F (µg) per unit exposed enamel surface area (cm^2).[11]

The equation to calculate the amount of F on the enamel in µg/cm^2 is as follows.

$$WF_{ON}(\mu g\ cm^{-2}) = \frac{W_F(\mu g)}{\text{exposed area}\,(cm^2)}$$

Assay of KOH-insoluble F

Subsequent to the KOH-soluble F estimation, the loosely bound F was removed from the enamel surface and the specimens were further processed for KOH-insoluble F estimation. The individual units were first rinsed with ultrapure water and blotted twice with blotting paper. An enamel microbiopsy was essential for KOH-insoluble F estimation and was performed using the method advocated by Kadoma et al.[12] A piece of adhesive tape with a 2 mm diameter window was placed on the surface of the enamel specimen. Disks 4 mm in diameter and 1 mm in thickness were punched out from glass-fiber filter paper (GF/B, GE Healthcare United Kingdom Ltd). 5 µL of 6 M hydrogen chloride (HCl) was pipetted onto the exposed enamel surface to etchfour successive enamel layers. After 30 seconds of etching, the HCl solution remaining on the enamel window was blotted twice with the filter paper, and all four disks were placed in 1 mL of ultrapure water. The water samples were then subjected to assays for F and calcium. The F concentrations were measured using the F-ion-selective electrode for KOH-soluble F.

The calcium concentrations were measured by placing a calcium-ion-selective electrode (Cornley Acculyte-5P M.C HI-TECH Co. Ltd) into a solution containing 0.5 mL of the sample, 0.5 mL of ultrapure water, and 128 µl of 0.1 M NaOH and 20 µL of TISAB (ISA, Thermo Scientific, Waltham Ma USA). The final aliquot volume was 1148 µL, with a pH of 7.0. The weight and volume of enamel removed by each acid etch and the corresponding F concentration was calculated using values of 2.95 for human enamel density and 37% for calcium content. We estimated the thickness of the enamel layer by dividing the enamel volume by the area of the exposed window (3.14 mm^2). The F concentration profile for the surface enamel was drawn using four continuous data points. The F concentration at 10 µm from the enamel surface was calculated by the interpolation of two data points at depths greater and <10 µm.

SEM Analysis

The randomly selected 16 (two from each temperature subgroup of group I and group II) specimens were analyzed under an SEM for surface structural changes.

All the specimens were remounted cylindrically on self-polymerizing resin, and the enamel surfaces were cross-sectioned with a hard tissue microtome to obtain a depth of 25–30 µm.

The sectioned enamel was mounted on stubs and coated in gold with the help of a gold coating machine. These gold coated units were mounted and analyzed under an SEM adjusted to 2000 volt, 30 nm resolution at 10,000 × and 2,500 × magnifications for the width of 5–10 µm.

Statistical Analysis

All the readings obtained were organized in an excel sheet and subjected to statistical analysis. Intergroup comparison was made using an unpaired *t*-test and intragroup comparison was done using one-way ANOVA univariate analysis. For all the tests, the level of significance was set at $p \leq 0.05$.

Results

The effectiveness of F was determined according to KOH soluble F, KOH insoluble F, and SEM analysis of the enamel surface.

KOH Soluble F

There was a significant difference in KOH soluble F levels at temperatures 25°, 37°, 50°, and 60°C for Fluor-Protector® and Embrace® varnish, respectively. Pairwise comparison of F for Fluor-Protector®, estimated in KOH solution, showed the maximum difference when the temperature increases from 25° to 37°C (Table 1).

Pairwise comparison of F for Embrace® varnish between different temperature groups, estimated in KOH solution, showed a significant difference when temperature increases from 25° to 50°C and 25° to 60°C (Table 2).

KOH Insoluble F

KOH insoluble F, estimated in ultrapure water by F ion-selective electrode for Fluor-Protector® and Embrace® varnish, showed statistically significant difference for group I, $F = 46.597$, $p = 0.001$ and group II, $F = 12.831$, $p = 0.001$, at temperatures of 25°, 37°, 50°, and 60°C, respectively.

Pairwise comparison between different temperature groups for KOH insoluble F, estimated in ultrapure water by F ion-selective electrode for Fluor-Protector® varnish, showed a statistically significant difference in F levels for an increase in temperature from 25° to 37°C, 37° to 50°C, and 37° to 60°C (Table 3).

Pairwise comparison between different temperature groups for KOH insoluble F, estimated in ultrapure water by F ion-selective

Table 1: Pairwise comparison between different temperature groups for F estimated in KOH solution by the ion-selective electrode for Fluor-Protector® varnish

				95% confidence interval	
(I) Temperature	*(J) Temperature*	*Mean difference (I-J)*	*p-value*	*Lower bound*	*Upper bound*
25°C	37°C	−9.90	0.016*	−18.31	−1.49
	50°C	−3.14	0.747	−11.55	5.27
	60°C	−6.19	0.214	−14.60	2.22
37°C	25°C	9.90	0.016*	1.49	18.31
	50°C	6.76	0.153	−1.65	15.17
	60°C	3.71	0.638	−4.69	12.12
50°C	25°C	3.14	0.747	−5.27	11.55
	37°C	−6.76	0.153	−15.17	1.65
	60°C	−3.04	0.764	−11.46	5.36
60°C	25°C	6.19	0.214	−2.22	14.60
	37°C	−3.71	0.638	−12.12	4.69
	50°C	3.04	0.764	−5.36	11.46

Post hoc Tukey test; *indicated significant at $p \leq 0.05$

Table 2: Pairwise comparison between different temperature groups for F estimated in KOH solution by the ion-selective electrode for Embrace® varnish

				95% confidence interval	
(I) Temperature	*(J) Temperature*	*Mean difference (I-J)*	*p-value*	*Lower bound*	*Upper bound*
25°C	37°C	−6.00	0.114	−12.99	0.99
	50°C	10.00	0.003*	3.00	16.99
	60°C	13.38	0.001*	6.38	20.37
37°C	25°C	6.00	0.114	−0.99	12.99
	50°C	16.00	0.001*	9.00	22.99
	60°C	19.38	0.001*	12.38	26.37
50°C	25°C	−10.00	0.003*	−16.99	−3.00
	37°C	−16.00	0.001*	−22.99	−9.00
	60°C	3.38	0.568	−3.61	10.37
60°C	25°C	−13.38	0.001*	−20.37	−6.38
	37°C	−19.38	0.001*	−26.37	−12.38
	50°C	−3.38	0.568	−10.37	3.61

Post hoc Tukey test; *indicated significant at $p \leq 0.05$

electrode for Embrace® varnish, showed a statistically significant difference in F uptake for the increase in temperature from 25° to 37°C, and the significant decrease of F uptake when the temperature was increased from 37° to 50°C and 37°–60°C, respectively (Table 4).

Comparison Between Fluor-Protector® and Embrace®

KOH soluble F (μg/cm^2) and KOH insoluble F, estimated (in ppm) in ultrapure water by F ion-selective electrode between Fluor-Protector® and Embrace® varnish at different temperatures showed statistically significant differences (Tables 5 and 6).

There was also a significant difference in the amount of calcium estimated in ultrapure water by calcium ion-selective electrode for Fluor-Protector® and Embrace® F varnish groups at different temperatures ($p < 0.05$) (Table 7).

Discussion

Topical F application in the form of varnishes may lead to the appreciable acquisition of F in both enamel and dentine samples. Especially, KOH-soluble F deposits on the surfaces of dental hard tissue can be increased, depending on the F concentration and composition of the F varnish applied.[13,14]

In recent studies, when the temperature of the F agent was increased prior to its application, there was a significant increase in F uptake on the enamel surface. Hence, four different temperature groups were used in the present study; 25°C was considered as room temperature, 37°C mimicked the human body temperature; 50°C was the maximum tooth enamel bearing temperature without pulpal damage, and 60°C was used to evaluate the changes in F uptake at elevated temperatures. Cost-benefit and caries preventive effect are both important determinants involved in the acceptance of varnish as an F delivery vehicle in both public and private practice.[15]

Subsequent to the application on the enamel surface, topical F reacts with hydroxyapatite crystals to form CaF_2-like material. When exposed to a strongly alkaline solution (1 M KOH), the former salt dissolves (Ca, F) with no detectable dissolution of the apatite structure. The F that leaches into the KOH solution in the first 24 hours is called KOH soluble F.[11] Topical F application also facilitates the incorporation of F into the enamel surface.

Table 3: Pairwise comparison between different temperature groups for KOH insoluble F estimated in ultrapure water by ion-selective Electrode for Fluor-Protector® varnish

(I) Temperature	*(J) Temperature*	*Mean difference (I-J)*	*p-value*	*95% confidence interval*	
				Lower bound	*Upper bound*
25°C	37°C	−916.400*	0.001*	−1235.06	−597.74
	50°C	321.000*	0.048	2.34	639.66
	60°C	254.400	0.157	−64.26	573.06
37°C	25°C	916.400*	0.001*	597.74	1235.06
	50°C	1237.400*	0.001*	918.74	1556.06
	60°C	1170.800*	0.001*	852.14	1489.46
50°C	25°C	−321.000*	0.048*	−639.66	−2.34
	37°C	−1237.400*	0.001*	−1556.06	−918.74
	60°C	−66.600	0.942	−385.26	252.06
60°C	25°C	−254.400	0.157	−573.06	64.26
	37°C	−1170.800*	0.001*	−1489.46	−852.14
	50°C	66.600	0.942	−252.06	385.26

Post hoc Tukey test; *indicated significant at $p \leq 0.05$

Table 4: Pairwise comparison between different temperature groups for KOH insoluble F estimated in ultrapure water by the ion-selective electrode for Embrace® varnish

(I) Temperature	*(J) Temperature*	*Mean difference (I-J)*	*p-value*	*95% confidence interval*	
				Lower bound	*Upper bound*
25°C	37°C	−268.700*	0.012*	−490.64	−46.76
	50°C	202.900	0.083	−19.04	424.84
	60°C	136.300	0.362	−85.64	358.24
37°C	25°C	268.700*	0.001*	46.76	490.64
	50°C	471.600*	0.001*	249.66	693.54
	60°C	405.000*	0.001*	183.06	626.94
50°C	25°C	−202.900	0.083	−424.84	19.04
	37°C	−471.600*	0.001*	−693.54	−249.66
	60°C	−66.600	0.850	−288.54	155.34
60°C	25°C	−136.300	0.362	−358.24	85.64
	37°C	−405.000*	0.001*	−626.94	−183.06
	50°C	66.600	0.850	−155.34	288.54

Post hoc Tukey test; *indicated significant at $p \leq 0.05$

This acquired F in the enamel surface is structurally bound F and cannot be removed by KOH treatment, and is known as KOH insoluble F.[11]

Studies conducted by Dijkman et al.,[16] Caslavaska,[17] and Retief et al.[18] have demonstrated a considerable amount of KOH soluble F on the enamel surface; 21–30 µgm/cm^2 for Duraphat (5%) and 53–60 µgm/cm^2 for Fluor-Protector®, respectively. The increase in KOH soluble F is a result of the exposed surface area of the enamel blocks and the F concentration of the varnish used in their studies. Barrancos,[19] and Mellberg and Loertscher,[7] reported that F absorption increases with an increase in room temperature, while Stookey and Stahlman[20] showed that F uptake from a solution could be increased by raising the temperature of the storage container by scrubbing. Putt et al.,[9] also reported an increase in both F and tin uptake when the temperature of stannous F solution was raised, with the most significant increase in F content observed at 65° and 85°C.

In the present study, as the temperatures were increased from 25° to 37°C, there was a marked rise in KOH soluble F for both Fluor-Protector® and Embrace® varnish groups, which was statistically significant. This result confirms that the change in temperature of the F varnish has an influence on the uptake of F onto the enamel surface. Also, both the experimental groups demonstrated the formation of KOH-soluble F, which consists of a CaF_2-like layer. This entity is usually formed on dental hard tissues, following the application of ionically bound Fs or in an F-rich environment. This deposit later dissolves in the saliva or the plaque layer during a drop in the pH, allowing F to diffuse into the underlying enamel.[21,22] Although F uptake increased significantly when the temperature was increased from 25° to 37°C, further increase in temperature from 37° to 50° and 60°C did not increase the F uptake. This can be attributed to the heat-volatile contents like ethanol and polyurethane base, which are contained in an F varnish. At temperatures 50°C and above, the structural change and distortion of the material alter the properties of the varnish, which prevents the release of F ions.

The difference in KOH insoluble F obtained at different temperatures for Fluor-Protector® and Embrace® varnishes confirm that the change in temperature of F varnish has an influence on the incorporation of F into the enamel (acquired or

Table 5: Difference in KOH soluble F (μg/cm^2) estimated in KOH solution by the ion-selective electrode between Fluor-Protector® and Embrace® varnish at different temperatures

				95% confidence interval of the difference			
Temperature	*Groups*	*Mean*	*Mean difference*	*Lower*	*Upper*	*t-value*	*p-value*
25°C	Fluor-Protector®	69.81	4.10	−2.22	10.41	1.363	0.190
	Embrace®	65.71					
37°C	Fluor-Protector®	79.71	8.00	1.54	14.46	2.600	0.018*
	Embrace®	71.71					
50°C	Fluor-Protector®	72.95	17.24	11.29	23.18	6.092	0.001*
	Embrace®	55.71					
60°C	Fluor-Protector®	76.00	23.67	18.31	29.03	9.277	0.001*
	Embrace®	52.33					

Independent *t*-test; *indicated significant at $p \leq 0.05$

Table 6: Difference in KOH insoluble F (in ppm) estimated in ultrapure water by the ion-selective electrode between Fluor-Protector® and Embrace® varnish at different temperatures

				95% confidence interval of the difference			
Temperature	*Groups*	*Mean*	*Mean difference*	*Lower*	*Upper*	*t-value*	*p-value*
25°C	Fluor-Protector®	1684.60	326.50	106.75	546.25	3.122	0.006*
	Embrace®	1358.10					
37°C	Fluor-Protector®	2601.00	974.20	742.23	1206.17	8.823	0.001*
	Embrace®	1626.80					
50°C	Fluor-Protector®	1363.60	208.40	10.62	406.18	2.214	0.040*
	Embrace®	1155.20					
60°C	Fluor-Protector®	1430.20	208.40	2.698	414.10	2.128	0.047*
	Embrace®	1221.80					

Independent *t*-test; *indicated significant at $p \leq 0.05$

Table 7: Difference in calcium estimated (μg/cm^2) in ultrapure water between Fluor-Protector® and Embrace® varnish at different temperatures

				95% confidence interval of the difference			
Temperature	*Groups*	*Mean*	*Mean difference*	*Lower*	*Upper*	*t-value*	*p-value*
25°C	Fluor-Protector®	0.044	−0.005	−0.003	−0.005	−2.111	0.049*
	Embrace®	0.049					
37°C	Fluor-Protector®	0.041	−0.005	−0.002	−0.006	−2.824	0.011*
	Embrace®	0.046					
50°C	Fluor-Protector®	0.043	−0.006	−0.05	−0.002	−14.520	0.001*
	Embrace®	0.049					
60°C	Fluor-Protector®	0.045	−0.005	−0.003	−0.004	−18.469	0.001*
	Embrace®	0.050					

Independent *t*-test; *indicated significant at $p \leq 0.05$

structurally bound F). Various studies[23–26] conducted on topical sodium F and acidulated phosphate F have also concluded that the average increase of F incorporation in the enamel was a direct result of the increase in temperature of the topical agent. Takagi et al.[27] also showed that enamel resistance to lesion formation increased with tooth-bound F content. Thus, a higher enamel F concentration is considered advantageous for caries prevention, as it resists enamel dissolution in an acidic environment. Although these structurally incorporated Fs prevent demineralization, they are least effective with respect to remineralization of an initial carious lesion.[28,29]

At all the different temperatures, both KOH soluble F and KOH insoluble F were at a statistically greater amount in the Fluor-Protector® group as compared to the Embrace® varnish group. This confirms the results of the previous studies conducted on these F varnishes when applied at room temperature.[16,18,23] Fluor-Protector® varnish, when applied, gets absorbed and penetrates in and onto the enamel surface, thus making it a better choice for topical F application.

The least values of calcium levels were detected in the solutions of the enamel biopsy obtained from the Fluor-Protector® group. It can, thus, be concluded that Fluor-Protector® was better at preventing the dissolution of enamel as compared to the Embrace®

varnish. There is strong evidence to suggest that Fluor-Protector® varnish effectively prevents calcium dissolution and increases the hardness of enamel.[30,31]

SEM analysis exhibited a fair number of 25–30 µm thick globular CaF_2-like precipitates at all temperatures in both the varnish groups. However, higher numbers of these precipitates were observed at 37°C in the Fluor-Protector® varnish group. Similar spherical globules of CaF_2 have also been observed in studies conducted by Attin et al.,[13] Cruz et al.,[32] and Jeng et al.[33] In general, these studies indicate that the main product of F treatment is the CaF_2-like deposits on the enamel surface. The contributions of these deposits to anticaries activity are well-documented.[34,35]

Conclusion

Fluor-Protector® provided better F uptake into and onto the human enamel and demonstrated a better potential for demineralization as compared to the Embrace® varnish. Topical F varnishes are most effective at 37°C, which is close to the human body temperature. Thus, the application of warm F varnish ensures more uptake of F onto the enamel surface for greater protection against dental caries. However, it must be noted that, unlike topical F gels and solutions, varnishes do not retain their F-releasing properties at higher temperatures.

Availability of Data and Material

Available on request from the corresponding author.

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