Assessment of Fluoride Release Pattern from Different Fluoride Varnishes – An in vitro Study

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Authors’ contributions

This work was carried out in collaboration among all authors. Author SS designed the study. Author AMB wrote the protocol. Author RK wrote the first draft of the manuscript. Author JMC managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i3330955

Received 18 September 2020
Accepted 22 November 2020
Published 10 December 2020

ABSTRACT

Background: Since the time they were introduced, researchers have put in endeavours to better the delivery of the fluoride in the fluoride varnishes. With numerous fluoride varnishes accessible in the market today, each product claims of superior and enhanced properties. In the recent years, significant differences have been reported in these products in terms of cumulative amount as well as the amount of fluoride released.

Aim: To determine the fluoride release pattern of four different calcium-phosphate based 5% sodium fluoride varnishes.

Materials and Methods: This in vitro study was conducted at Department of Pedodontics and Preventive Dentistry, Christian Dental College, Ludhiana, Punjab, India for 6 months. 75 non-carious premolar teeth were randomly divided into five groups and coated with a different coloured nail varnish except for a 3mm X 3mm window on the labial surface of the crown. Four groups received fluoride varnish coating on this window while the fifth group served as the control. The various fluoride varnishes tested included MI VarnishTM(containing CPP-ACP), Clinpro™ White

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Varnish (containing f-TCP), Embrace™ Varnish (containing xylitol coated calcium and phosphate) and Enamel Pro® Varnish (containing ACP). Thereafter, the 15 teeth of each group were placed together in a plastic container containing 50ml of artificial saliva. After 1 day, 1 month, 3 months and 6 months, the teeth were placed in a new plastic container containing 50ml of fresh artificial saliva while the previous saliva sample was assessed for the fluoride released in it through the SPADNS Method. The data was compiled by using Microsoft Excel 2010 SPSS version (SPSS Inc., Chicago, Illinois, USA).

**Results:** Clinpro™ White Varnish exhibited the greatest cumulative fluoride release while MI Varnish™ exhibited the greatest substantivity at the end of 6 months. Three of the tested varnishes released increasing amounts of fluoride up to 3 months, thereafter, the values began to decline. Meanwhile, Embrace™ Varnish released increasing amount of fluoride only up to one month, thereafter the values began to decline.

**Conclusion:** The varying excipient ingredients could be held accountable for the different fluoride release pattern of the studied on calcium phosphate based 5% sodium fluoride varnishes.

**Keywords:** MI Varnish™, Clinpro™ white varnish; Embrace™ varnish; enamel pro® varnish; SPADNS method.

**ABBREVIATIONS**

CPP-ACP: Casein phosphopeptide-amorphous calcium phosphate.

f-TCP : Functionalized-tricalcium phosphate.

ACP : Amorphous calcium phosphate.

**1. INTRODUCTION**

Biofilm (plaque) – induced acid demineralisation of enamel or dentin, mediated by saliva leads to dental caries [1]. It’s an infectious disease modified by dietary carbohydrates which are utilized by specific acidogenic bacteria for the production of acids which commence the breakdown of tooth structures [2].

Upon reviewing the global burden of diseases, Kassebaum et al. found that untreated caries affects around 2.5 billion people in the permanent dentition and around 573 million children in the primary dentition [3]. Furthermore, considering the Indian scenario, dental caries is prevalent in nearly 50% of the five-year old and twelve-year old population group [4]. According to the systematic review conducted by Ganesh et al. on early childhood caries, it was found that this caries pattern affects 49.6% of the Indian children [5].

A paradigm shift in the management of carious lesions from an operative-restorative approach towards a prevention-remineralisation concept has emerged in the recent decades on grounds of better understanding of the ultrastructure and the dynamic nature of enamel caries [6]. Moreover, in view of Covid-19 pandemic, Al-Halabi et al. critically reviewed the caries management alternatives in the post COVID-19 period and recommended the non-aerosol generating procedures must become a necessity with utmost priority given to preventive procedures [7].

Fluoride is the most frequently used remineralising agent [8]. However, a significant shortcoming associated with the use of topical fluoride agents is their comparatively superficial effect on dental enamel which is due to their relatively lesser duration during which they remain in contact with the tooth. For the purpose of increasing the fluoride actions in mouth, a novel coating method was put forward by Schmidt in 1964. Due to this method, a fluoride lacquer which was coated on the teeth, released fluoride ions at high concentrations in the presence of moisture in the oral cavity for few hours. Consequently, the preparation exerted a concentrated fluoridation effect as well as a deeper action, an action that was previously unattainable in the oral cavity. As a result, varnishes have became the treatment of choice for preventing dental caries [9].

Since fluoride varnishes contain high concentrations of fluoride, they lead to the formation of non-stoichiometric calcium fluoride [10] that precipitate on the sound enamel surface, biofilm, pellicle as well as enamel porosities. The adsorption of HPO42- serves as the limiting factor for the rate of dissolution of these globules. As HPO42- is lost under acidic pH conditions, CaF2 is allowed to dissolve and consequently calcium and fluoride are released. This fluoride contributes to the fluoride pool present at the enamel fluid [11].
Shahmoradi et al. noted that the sole presence of fluoride is not adequate for the purpose of preventing demineralisation and redepositing minerals into hydroxyapatite crystals [6]. In order to form one unit cell of fluoroapatite \([\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2]\), two fluoride ions, ten calcium ions and six phosphate ions are needed. Therefore, the availability of calcium and phosphate ions serves as the limiting factor for total remineralisation to occur following the topical application of fluoride ions [12]. In view of this, many manufacturers have added calcium and phosphate ions to fluoride varnishes for the purpose of increasing their efficacy [13].

However, the formation of poorly soluble calcium fluoride phosphate phases is the disadvantage that accompanies the addition of \(\text{Ca}^{2+}\) and \(\text{PO}_4^{2-}\) salts to \(\text{F}^-\) ions. These phases can form either in the material package during storage or in the saliva following the application of the material. Such poorly soluble phases bring about a reduction in the quantity of bioavailable \(\text{F}^-\) ions which are in turn an absolute requirement for the formation of \(\text{CaF}_2\) globules on the tooth surface [6].

Numerous propositions have been made with the motive of stabilizing the fluoride, calcium and phosphate ions in varnishes during the storage of the material including stabilizing the \(\text{Ca}^{2+}\) and \(\text{PO}_4^{2-}\) ions by casein phosphopeptide, preventing the reaction between \(\text{Ca}^{2+}\) and \(\text{F}^-\) ions through a protective fumaric acid barrier and also by coating the \(\text{Ca}^{2+}\) and \(\text{PO}_4^{2-}\) with xylitol [6].

The \(\text{F}^-\) available for preventing caries following the application of fluoride varnish is denoted by the salivary fluoride levels [14]. Additionally, once a week for three weeks consecutively, three months and six months intervals are the recommended time intervals for administrating fluoride varnish to bring about reversal of non-cavitated carious lesions as well as prevention of caries amongst individuals at risk of developing caries as per ADA evidence-based clinical recommendations [15,16]. Literature search did not reveal any study that compared the fluoride release pattern of different calcium phosphate based 5% NaF varnishes for six months.

Therefore, the present study was undertaken to compare different calcium-phosphate containing varnishes, i.e., – MI Varnish\textsuperscript{TM} containing casein phosphopeptide-amorphous calcium phosphate, Clinpro\textsuperscript{TM} White Varnish containing functionalized tricalcium phosphate, Embrace\textsuperscript{TM} Varnish containing xylitol coated calcium and phosphate and Enamel Pro\textsuperscript{®} Varnish containing amorphous calcium phosphate in terms of their fluoride release into artificial saliva after one day, one month, three months and six months following the varnish application.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Once approval was obtained from the Research Committee, seventy five premolar teeth which were extracted for orthodontic purposes were obtained with the criteria for tooth selection being intact crown and caries free teeth. Following this, the teeth were thoroughly cleaned with ultrasonic scaler and polished with pumice slurry and rubber prophylaxis cup and stored in normal saline. Then, the teeth were randomly divided into five groups with each group comprising of 15 teeth.

2.2 Preparation of Samples

A different coloured nail varnish was used to coat the tooth surfaces except of a 3mm X 3mm window on the facial surface of the crown.

2.3 Intervention

The 3mm X 3mm window on the facial surface of the crown was coated with MI Varnish\textsuperscript{TM} in Group I teeth; with Clinpro\textsuperscript{TM} White Varnish in Group II teeth; with Embrace\textsuperscript{TM} Varnish in Group III teeth and with Enamel Pro\textsuperscript{®} Varnish in Group IV teeth as shown in Table 1 and Figs. 1-2.

This window did not receive any fluoride treatment in the Group V teeth which served as the control. All the 15 teeth of each group were placed in a plastic container containing 50ml of artificial saliva as shown in Fig. 3.

This saliva was changed sequentially at one day, one month, three months and six months post-application of the fluoride varnish. The saliva sample from the previous day was taken to the laboratory for estimation of fluoride release.

2.4 Fluoride Release Measurement for One Day Samples

10 ml of artificial saliva sample was pipetted into a cuvette. 2 ml of SPADNS reagent was added to the 10 ml of artificial saliva. This cuvette was
then shaken for one minute. Thereafter, the cuvette was left still for two minutes. Finally, the cuvette was placed in the pre-adjusted spectrophotometer which is shown in Fig. 4. (fluoride value at 0.00 mgF/L at wavelength of 570 nm).

Fig. 1. Calcium-phosphate based 5% NaF varnishes tested in the study

Fig. 2. Covering of tooth surfaces by a nail varnish except for a 3mm X 3mm window on the facial surface of the crown where the coating of the varnish was applied
Fig. 3. Immersion of all 15 teeth of a group in plastic containers containing 50ml of artificial saliva

Fig. 4. Spectrophotometer and SPADNS reagent employed for determination of amount of fluoride released
Table 1. Detailed information of various fluoride varnishes tested

<table>
<thead>
<tr>
<th>MATERIAL</th>
<th>MANUFACTURER</th>
<th>MATERIAL TYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI Varnish™</td>
<td>GC CORPORATION (LOT 1606032)</td>
<td>5% Sodium fluoride varnish containing casein phosphopeptide-amorphous calcium phosphate</td>
</tr>
<tr>
<td>Clinpro™ White Varnish</td>
<td>3M ESPE (LOT NA47317)</td>
<td>5% Sodium fluoride varnish containing functionalized tricalcium phosphate</td>
</tr>
<tr>
<td>Embrace™ Varnish</td>
<td>PULPDENT Corporation (LOT 171006)</td>
<td>5% Sodium fluoride varnish containing xylitol coated calcium and phosphate</td>
</tr>
<tr>
<td>Enamel Pro® Varnish</td>
<td>Premier Dental Products Company</td>
<td>5% Sodium fluoride varnish containing amorphous calcium phosphate</td>
</tr>
</tbody>
</table>

The reading displayed on the spectrophotometer was directly taken as the amount of fluoride released.

2.5 Fluoride Release Measurement for One month, Three months and Six Months Samples

Owing to the high fluoride values, 1 ml of artificial saliva was pipetted into a 100 ml graduated cylinder. In the same cylinder, 100 ml of distilled water was added. The cylinder was then shaken for one minute. Then, 10 ml of liquid from the cylinder was pipetted into another cuvette. 2 ml of SPADNS reagent was added to this cuvette. The cuvette was then shaken for one minute. Thereafter, the cuvette was left still for two minutes. Finally, the cuvette was placed in the pre-adjusted spectrophotometer (fluoride value at 0.00 mgF⁻/L at wavelength of 570 nm).

Since the reading obtained on the spectrophotometer displayed the amount of fluoride released by the diluted sample of artificial saliva, therefore, the total amount of fluoride released was calculated by substituting into Equation 1.

Fluoride mg/L = 50 A/V.

where,

A = Amount of fluoride (mg) measured by spectrophotometry.
V = volume of the specimen (ml) [17].

2.6 Statistical Analysis

The data was compiled by using Microsoft Excel 2010 SPSS version (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics included computation of the means and standard deviations. The statistical test applied for the analysis was repeated measure Analysis of Variance followed by post-hoc analysis. The confidence interval and p-value were set at 95% and ≤ 0.05 respectively.

3. RESULTS

Upon comparison, it was found that one day post the application of the fluoride varnish, Clinpro™ White Varnish released the most amount of fluoride while Enamel Pro® Varnish released the least amount. One month post the application, Clinpro™ White Varnish continued to release the most amount of fluoride while MI Varnish™ released the least amount. Three months post the application, Clinpro™ White Varnish yet again released the maximum amount of fluoride while Embrace™ Varnish released the least amount. Six months post the application, MI Varnish™ released the greatest amount of fluoride while Embrace™ Varnish continued to release the least amount. Additionally, the control group did not release any detectable amount of fluoride at any of the time-intervals as shown in Table 2 and Graph 1.

In terms of cumulative release, it was noted that Clinpro™ White Varnish exhibited the greatest cumulative release of fluoride while Embrace™ Varnish released the lowest cumulative amount of fluoride as shown in Graph 2.

In terms of substantivity, i.e., ability to release fluoride over a prolonged period of time, it was observed that MI Varnish™ exhibited the maximum substantivity since it had released the maximum amount of fluoride at the end of 6 months, while the fluoride got depleted from Embrace™ Varnish at a relatively greater rate as shown in Graph 3.

In terms of pattern of fluoride release, it was found that MI Varnish™, Clinpro™ Varnish and Enamel Pro® Varnish continued to release
increasing amounts of fluoride consecutively for the duration of three months while Embrace™ Varnish continued to release increasing amounts of fluoride only for the duration of one month, thereafter, it consecutively released decreasing amounts as shown in Graph 4.

Repeated Measures ANOVA was employed for both inter-group and intra-group comparisons and it revealed a highly statistically significant difference by all the varnishes at any particular time period and also by any particular varnish at different time-intervals in majority of the cases.

Graph 1. Mean fluoride released by the tested varnishes at different time-intervals
Graph 2. Cumulative fluoride released for the duration of 6 months with Clinpro™ White Varnish releasing the greatest amount and Embrace™ Varnish releasing the lowest amount.

Graph 3. Substantivity of the tested varnishes with MI Varnish™ showcasing the most substantivity and Embrace™ Varnish showcasing the least by the end of 6 months.
Graph 4. Pattern of fluoride released. With the exception of Embrace™ Varnish, all the other varnishes continued to release increasing amounts for the duration of 3 months consecutively.

Table 2. Mean ± S.D. of amount of fluoride released by various groups at different time intervals

<table>
<thead>
<tr>
<th>Test Group</th>
<th>Time Interval</th>
<th>Mean ± S.D. of fluoride released in terms of spectrophotometer readings in mgF⁻/L without dilution</th>
<th>Mean ± S.D. of fluoride released in terms of spectrophotometer readings in mgF⁻/L with dilution</th>
<th>Mean ± S.D. of fluoride released after applying the formula ( \text{mgF}⁻/L = 50 \times \text{A/V} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>1 Day</td>
<td>1.52 ± 0.007</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group II</td>
<td>1 Day</td>
<td>1.76 ± 0.026</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group III</td>
<td>1 Day</td>
<td>1.52 ± 0.000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group IV</td>
<td>1 Day</td>
<td>1.35 ± 0.010</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group V</td>
<td>1 Day</td>
<td>0.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group I</td>
<td>1 Month</td>
<td>-</td>
<td>1.29 ± 0.030</td>
<td>6.45 ± 0.150</td>
</tr>
<tr>
<td>Group II</td>
<td>1 Month</td>
<td>-</td>
<td>1.52 ± 0.020</td>
<td>7.6 ± 0.100</td>
</tr>
<tr>
<td>Group III</td>
<td>1 Month</td>
<td>-</td>
<td>1.34 ± 0.019</td>
<td>6.71 ± 0.096</td>
</tr>
<tr>
<td>Group IV</td>
<td>1 Month</td>
<td>-</td>
<td>1.44 ± 0.044</td>
<td>7.2 ± 0.223</td>
</tr>
<tr>
<td>Group V</td>
<td>1 Month</td>
<td>0.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group I</td>
<td>3 Months</td>
<td>-</td>
<td>1.39 ± 0.010</td>
<td>6.95 ± 0.050</td>
</tr>
<tr>
<td>Group II</td>
<td>3 Months</td>
<td>-</td>
<td>1.61 ± 0.014</td>
<td>8.05 ± 0.070</td>
</tr>
<tr>
<td>Group III</td>
<td>3 Months</td>
<td>-</td>
<td>1.31 ± 0.015</td>
<td>6.55 ± 0.079</td>
</tr>
<tr>
<td>Group IV</td>
<td>3 Months</td>
<td>-</td>
<td>1.46 ± 0.007</td>
<td>7.3 ± 0.035</td>
</tr>
<tr>
<td>Group V</td>
<td>3 Months</td>
<td>0.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group I</td>
<td>6 Months</td>
<td>-</td>
<td>0.70 ± 0.031</td>
<td>3.5 ± 0.158</td>
</tr>
<tr>
<td>Group II</td>
<td>6 Months</td>
<td>-</td>
<td>0.57 ± 0.014</td>
<td>2.85 ± 0.070</td>
</tr>
<tr>
<td>Group III</td>
<td>6 Months</td>
<td>-</td>
<td>0.39 ± 0.021</td>
<td>1.95 ± 0.106</td>
</tr>
<tr>
<td>Group IV</td>
<td>6 Months</td>
<td>-</td>
<td>0.59 ± 0.015</td>
<td>2.95 ± 0.079</td>
</tr>
<tr>
<td>Group V</td>
<td>6 Months</td>
<td>0.00</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

4. DISCUSSION

The present study assessed the fluoride release pattern of four differently composed calcium-phosphate based 5% NaF varnishes. It was the first of its kind to evaluate the amount of F⁻ released for a period as long as 6 months. The results of the study showed that the tested
varnishes differed in terms of fluoride release pattern, cumulative fluoride release and substantivity. Highly statistically significant differences were noted pertaining to the amount of fluoride released by any particular fluoride varnish at different time-intervals and also in relation to fluoride released at any particular time period by all the varnishes in majority of the cases.

With the exception of Embrace™ Varnish, all varnishes continued to release increasing amounts of fluoride consecutively for the duration of 3 months, thereafter, the values gradually began to decline as shown in Table 2 and Graph 1. Since the present study was the first of its kind to compare the varnishes for a span of 6 months, the results obtained could not be compared with any other study.

Clinpro™ White Varnish released the highest cumulative fluoride while Embrace™ Varnish released the lowest as shown in Graph 2. A study conducted by Mohd Said et al. in 2017 found similar results, however, their assessment parameter was different in that they compared the varnishes in terms of their remineralisation potential [18]. The superior performance of Clinpro™ White Varnish can be credited to the functionalizing of beta-tricalcium phosphate with organic/inorganic molecules. This offers dual advantages of creation of a barrier that aids to prevent premature fluoride-calcium reactions as well as ensuring targeted delivery [19].

The greatest substantivity was exhibited by MI Varnish™ as shown in Graph 3. This could be attributed to the depletion of fluoride ions in the Clinpro™ White Varnish or superior stabilization of Ca<sup>2+</sup>, PO<sub>4</sub><sup>2-</sup> and F<sup>-</sup> ions in solution by CPP component in the MI Varnish [20]. However, further studies are needed to truly attest this reasoning.

The fastest depletion of fluoride ions was noted in Embrace™ Varnish as shown in Graph 4. Similar to the observations in our study, Milburn et al. observed that inclusion of xylitol coated calcium and phosphate did not aid in sustaining high fluoride release over time [21].

Enamel Pro<sup>®</sup> Varnish demonstrated an initially slow fluoride release pattern which improved over time. The high inorganic phosphate content can be held responsible for this phenomenon since it is largely believed to be counterproductive in formation of loosely bound fluoride reservoirs [22].

The strength of the present study lies in its time periods since they match with the recommended time-intervals for recall fluoride varnish applications for management of non-cavitated carious lesions as well as prevention of caries amongst individuals at moderate to high risk for developing caries [15,16]. Since it is now gathered that Clinpro™ White Varnish exhibits rapid and high amount of fluoride release, it can be administered to caries-active children while MI Varnish™ can be administered to caries-prone children on the grounds of its slow and prolonged fluoride release pattern.

The limitations of the study were that the intra-oral conditions could not be fully simulated. There is more rapid fall in fluoride level of saliva intra-orally. Moreover, the human saliva is subjected to regular fluctuations in temperature, pH and protein content [14]. Future studies are recommended to assess the tested varnishes by employing an “in-vivo” design.

5. CONCLUSION

The varnishes available in the market today differ largely in terms of their fluoride release pattern, the total amount of fluoride released and the duration of fluoride released. A detailed knowledge of such characteristics will support future clinical studies since we will be hugely relying on preventive measures and non-aerosol generating procedures for instilling quality dental care in the post COVID-19 era.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

As per international standard or university standard, Participants’ written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).
ACKNOWLEDGEMENT

We duly acknowledge the efforts of the team at Environmental Engineering Department, Guru Nanak Dev Engineering College for helping us with our research work. We are also very grateful to Dr. Vivek for helping us with our statistical work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/62782