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Research Article

In Vitro Fluoride Release Potential of Preventa and Enamel Pro Fluoride Varnishes

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ABSTRACT

Objectives: This study sought to compare the fluoride release potential of Enamel pro and V-Varnish fluoride varnishes in vitro. **Methods:** In this in vitro, experimental study, V-Varnish (Korea, Vericom) , and Enamel Pro(USA, Premier) varnishes were each evaluated in 20 samples. The varnishes were applied on celluloid matrix bands such that the entire band was coated with the varnish. The bands were then immersed in 25 mL of deionized distilled water. They were removed from the water and rinsed with 5 mL of deionized distilled water at one, four, 24 and 72 hours and immersed in a fresh solution. The amount of fluoride released from the varnishes was measured by potentiometry and reported in ppm. Data were analyzed using SPSS version 20 via Shapiro Wilk test, repeated measures ANOVA followed by Bonferroni test and independent samples t-test. **Results:** Fluoride release rate significantly changed over time in both Enamel Pro and V-Varnish groups (both $P<0.001$). Enamel pro showed higher fluoride release at all time points than the first hour ($P<0.001$ for all three time points). After a relative increase at 4 hour, there was a decrease in release during the time in a way that the decrease in release was significant during the time ($P<0.001$ for all three time points). In V-Varnish group the fluoride release at 1 and 24 hour was not significant ($p=1.00$). other comparisons were significant ($p< 0.001$ in each item) **Conclusion:** The amount of fluoride release of V-Varnish was significantly higher than that of Enamel pro over 24 hours period and V-Varnish seems to be more effective as a fluoride varnish for professional application.

Keywords: Fluoride Release; Fluoride Varnish; Enamel Pro; V-Varnish

INTRODUCTION

Dental caries is defined as demineralization of calcified tooth structure and degradation of its organic contents [1]. It is among the most common human diseases and despite advances in caries prevention, it remains a common preventable disease worldwide [2]. The prevalence of caries has significantly decreased in developing countries; however, it is still

considerably high in developing countries. Prevention is the most efficient cost-effective approach for control of dental caries [3]. Increasing the resistance of enamel to acidic products is among the most important objectives of caries prevention programs [4]. Fluoride can greatly help in this respect since its role in caries prevention has been acknowledged for over 50 years [5]. Fluoride present in dental plaque can penetrate into the enamel of freshly erupted teeth and cause enamel remineralization and decrease the susceptibility of tooth structure to caries. Tooth structure remineralized in presence of fluoride contains high concentrations of fluorapatite, which confers further resistance to remineralized tissue against acid attacks [3].

It has been documented that frequent application of fluoride can significantly prevent dental caries in children and adults [6]. Fluoride can be used in many different ways such as fluoridation of drinking water, topical application of fluoride, taking fluoride tablets and using fluoridated tooth pastes, fluoride mouthwashes and fluoride varnishes [7]. Drinking water containing adequate amount of fluoride can prevent dental caries by 50-59% [3]. However, tap water in most parts of Iran does not contain adequate amount of fluoride. Thus, fluoride needs to be provided from other sources such as toothpastes, gels and fluoride varnishes. It has been documented that continuous application of fluoride in different ways serves as a prophylaxis against caries [8].

Clinical studies showed that, fluoride varnish can decline caries rate 5 to 25 percent. It has been reported that fluoride reservoir on the tooth surface is depended on the amount of fluoride ions released from fluoride products.(13) this issue is the cause of growing studies on the subject of amount and timing of fluoride release of different products.(11,15,16) as with prospering technologies new varnishes introduced to the market, that their fluoride release characteristics is still unknown. Application of fluoride varnishes and gels is highly effective for prevention and control of caries. Also, evidence shows that use of fluoride varnishes has advantages such as easy use, low risk of swallowing of high amounts of fluoride, high patient cooperation, not requiring professional prophylaxis and cleaning and not requiring strict isolation. Thus, application of fluoride varnishes is a suitable method of caries prevention.

Considering the gap of information on fluoride release potential of Enamel Pro and Preventa varnishes, this study aimed to compare the fluoride release potential of Enamel Pro and Preventa fluoride varnishes in vitro. The null hypothesis was that the fluoride release potential of Preventa and Enamel Pro varnishes would not be significantly different.

MATERIAL & METHODS

This in vitro experimental study was conducted on Enamel Pro and V-Varnishe. Sample size was calculated to be 20 in each group (total of 40) assuming type one error of 1.96, type two error of 0.2 and power of 0.80 according to a study by Comar et al [9].

Table 1 shows the constituents and properties of the two varnishes used in this study.

Sample preparation:

A total of 20 celluloid matrix bands measuring 20×40 mm were completely coated with Enamel Pro (Premier, USA) and another 20 with V-Varnish (Korea, Vericom) varnishes using similar micro-brushes by one operator via eight back and forth motions. To standardize the amount of varnish applied on each band, each band was weighed before and after coating with the varnish using a digital scale (AL-104; Acculab, Sartorius group, Goettingen, Germany) with 0.0001 g accuracy. The difference in weight of each band before and after varnish application indicated the weight of varnish used for coating of the bands. All bands were standardized in terms of the weight of varnish used for their coating as such.

Coated bands were immersed in 25 mL of deionized distilled water at 37°C and 95% humidity in a cold incubator (Aria Sanat, Tehran, Iran). They were removed from the water after one, four, 24 and 72 hours, rinsed with 5 mL of deionized distilled water and placed in fresh

water; 5 mL of the 30 mL final solution was removed by graded pipette and mixed with 0.5 mL of total ionic strength adjustment buffer (Merck, Darmstadt, Germany) for measurement of fluoride concentration. This solution has optimal pH for activity of fluoride electrode and adjustment of ionic strength. The total ionic strength adjustment buffer contained 0.2 g/L CDTA with the formulation of O-2-trans di-amino cyclohexane-N,N,N,N, tetraacetic acid (C14H2N2O8H2O), 57 mg/L glacial acetic acid (CH2COOH) and 85 g/L sodium chloride. Its pH was adjusted to 5.5 by addition of sodium hydroxide. The amount of fluoride released in the solutions was measured using ion analyzer (Crison Instrument, s.a., Barcelona, Spain). This device has two channels for measurement of pH and ionic concentration of solutions. The first channel, used for measurement of pH, has the ability for 1,2 and 3-point calibration with reference solutions. It measures the pH with 0.01 accuracy. The second channel is used for measurement of ionic concentration using potentiometry and has 5-point calibration ability with 0.1, 0.01, 0.001, 0.0001 and 0.00001 standards for measurement of fluoride concentration in mol/L and mg/L. The second channel of this device was used in our study.

Statistical analysis

The mean amount of released fluoride and its standard deviation were calculated for the two groups at different time points. The Shapiro Wilk test was used to assess the normal distribution of data. Repeated measures ANOVA was used for the comparison of the two groups and Bonferroni test was used for pairwise comparisons of fluoride release potential of each varnish at different time points.

Independent samples t-test was applied for the comparison of the fluoride release potential of the two varnishes at each time point. because of significant interactions $P<0.05$ was considered statistically significant.

RESULTS

Table 2 shows the results of Shapiro-Wilk test for assessment of normal distribution of data. As shown in Table 2, data were normally distributed at all time points ($P>0.05$) and thus, parametric tests were applied.

Table 3 shows statistical indices of fluoride released in the two groups at different time points. According to the interaction effect of time of fluoride measurement and type of varnish on the amount of released fluoride ($P<0.001$) as shown in Figure 1, the trend of reduction or increase in fluoride release was not the same at different time points for each varnish group. Thus, comparisons were made at each time point (between the two varnishes) and also for each varnish over time.

To comprise fluoride release potential of each varnish at different time points:

Repeated measures ANOVA showed significant change in fluoride release of Enamel Pro and PreventaV-Varnish over time ($P<0.001$ for both).

Fluoride release rate significantly changed over time in both Enamel Pro and V-Varnish groups (both $P<0.001$). Enamel pro showed higher fluoride release at all time points than the first hour ($P<0.001$ for all three time points). After a relative increase at 4 hour, there was a decrease in release during the time in a way that the decrease in release was significant during the time ($P<0.001$ for all three time points). In V-Varnish group the fluoride release at 1 and 24 hour was not significant ($p=1.00$). other comparisons were significant ($p< 0.001$ in each item). Increase in fluoride release at 4 hour was seen in comparison to the first hour and decrease of that during the time up to 72 hours was seen in V-Varnish but in V-Varnish the rate of decrease was more Enamel pro.

Comparison of fluoride release potential of the two varnishes at each time point: Independent samples t-test showed that there was a significant difference in the amount of fluoride release at all time points, between the two fluoride vanishes in a way that at 4 and 24 hour, the amount of fluoride release in V-Varnish was more than Enamel pro. ($P<0.001$ for all three time points) but at 72 hour, the amount of fluoride release of Enamel pro was more than V-Varnish.

Table 1. Constituents and properties of the two varnishes used in this study

Varnish	Constituents	Properties
Enamel Pro	Rosin, ethanol, 5% sodium fluoride, sodium phosphate and dehydrated calcium sulfate	It sets in presence of saliva and releases fluoride for 6-8 hours. It can be used for both primary and permanent teeth.
V-Varnish	Sodium fluoride 5%, Xylitol, TCP (trichlropane), enanol 2%	It can be used for both primary and permanent teeth.

Table 2. Results of Shapiro-Wilk test for assessment of normal distribution of data

Time point	Varnish	Statistic	Degree of freedom	P value
1 hours	Enamel Pro	0.906	20	0.53
	V-Varnish	0.966	20	0.675
4 hours	Enamel Pro	0.947	20	0.321
	V-Varnish	0.950	20	0.369
24 hours	Enamel Pro	0.947	20	0.838
	V-Varnish	0.909	20	0.057
72 hours	Enamel Pro	0.965	20	0.637
	V-Varnish	0.979	20	0.915

Table 3. Amount of fluoride released in the two groups (n=20) at different time points

Time/Material		Num	Mean	Standard deviation	95% confidence interval		Maximum	Minimum
					Lower bound	Upper bound		
1 hour	Enamel Pro	20	7550.4253	301.62267	6919.1218	8181.7288	10262.01	5944.97
	V-Varnish	20	11615.3656	224.74743	11144.9638	12085.7674	13314.99	9912.65
4 hours	Enamel Pro	20	11217.1347	106.17173	10994.9147	11439.3547	12262.28	10427.03
	V-Varnish	20	18666.7507	129.56878	18395.5601	18937.9413	19977.44	17821.08
24 hours	Enamel Pro	20	9453.2314	94.53581	9255.3656	9651.0971	10324.77	8601.20
	V-Varnish	20	11376.1007	395.41045	10548.4971	12203.7043	16532.53	8965.62
72 Hours	Enamel Pro	20	6835.1222	156.53522	6507.4902	7162.7542	7858.72	5323.89
	V-Varnish	20	3815.3399	115.95278	3572.6479	4058.0318	4801.83	2766.16

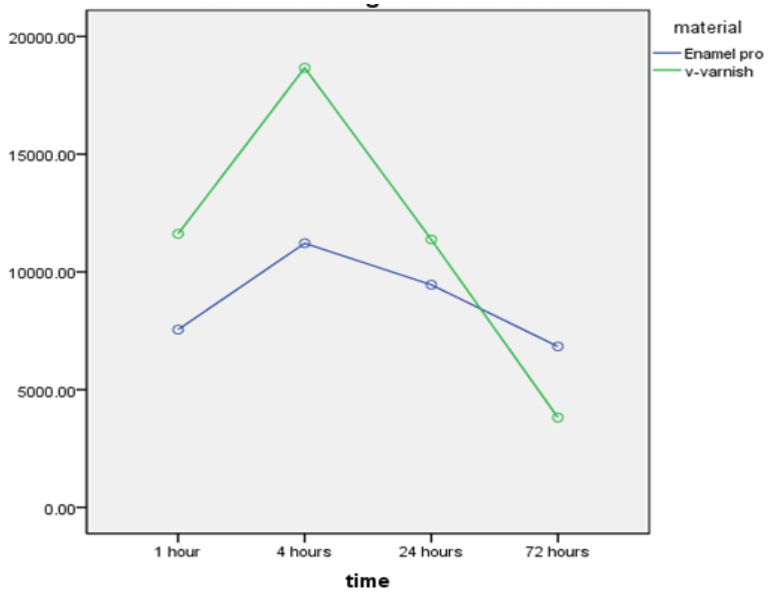

Figure 1. Trend of fluoride release of Enamel Pro and V-Varnish at different time points

DISCUSSION

This in vitro study evaluated the fluoride release potential of V-Varnish and Enamel Pro varnishes and showed that fluoride release rate significantly changed over time in both groups of varnishes and the amount of fluoride release at 1 hour to 24 hour in V-Varnish was more than Enamel pro but at 72 hour the amount of fluoride release in Enamel pro was more than V-Varnish.

Although both varnishes had the same concentration of fluoride in their composition, but the amount of fluoride release at different times between two varnishes was significantly significant.

According to the result of the study,V-Varnish has more amount of fluoride release than Enamel pro up to 24 hour and at 72 hour , the result was contradictory.

The efficacy of fluoride for decreasing dental caries has been well documented [10] and it has been reported that formation of fluoride reservoirs on the tooth surface depends on the amount of fluoride released from the product Greater fluoride release may eventually result in higher uptake of fluoride by the enamel [9].

The difference in fluoride release of the two varnishes in our study may be due to different resin carriers or additives used by the manufacturers, which might affect the fluoride release potential. Another possible explanation is presence of amorphous calcium phosphate in the composition of Enamel Pro.

Cochrane et al. [11] (2014) reported that fluoride varnish has a short life of about 24 hours in the oral cavity after application and is soon washed off the teeth as the result of movements of the tongue and cheeks, saliva flow, mastication and tooth brushing. Therefore, increase in fluoride release after 24 hours in Preventa varnish is not clinically beneficial for patients.

Cochrane et al, [11] (2014) assessed the release of calcium, inorganic phosphate and fluoride in MI, Clinpro White, Enamel Pro, Bifluoride 5 and Duraphat varnishes and reported significant differences in fluoride release in the first 24 hours following the application of each varnish. Jablonowski et al, [12] (2012) measured the amount of fluoride released from Enamel Pro, Duraphat and Varnish XT and reported that the fluoride release curve of Enamel Pro and Varnish XT was significantly different from that of Duraphat varnish. Also, they showed that Enamel Pro had the highest rate of fluoride release at all time points, which was in agreement with our findings. Ritwik et al, [13] (2012) compared the fluoride release of Premier Enamel Pro, Colgate PreviDent, Omni Varnish and Omni Varnish XT during 48 hours after their application and demonstrated that they all had different fluoride release curves. Premier Enamel Pro showed the highest rate of fluoride release at all time points, which was in line with our results. Bolis et al. [14] (2015) compared the fluoride uptake by the enamel and its release rate into the artificial saliva and showed the highest uptake of fluoride at four hours following the application of Fluor Protector S and Enamel Pro, respectively. Fluoride uptake had no significant association with its release rate. MI varnish had the highest rate of fluoride release in the first four hours following its application. In our study, Enamel Pro showed the highest rate of fluoride release at four hours. Lipert [15] (2014) quantified the release of fluoride from sodium fluoride varnishes in acidic conditions. The results showed that fluoride release potential of different varnishes depended on their decomposition factors and was significantly different. Enamel Pro and Nupro showed the highest fluoride release. Cochrane et al, [11] (2014) showed that the fluoride release from all tested products in vitro had an ascending trend from hour one to 168. However, in the study by Ritwik et al, [13] (2012) fluoride release from all products had a descending trend from one to 48 hours. Ritwik et al, [13] (2012) compared fluoride release of different varnishes during 48 hours following their application and reported that the fluoride release curve of the varnishes was significantly different.

Also, Colgate Prevident, Enamel Pro and Omni Varnish showed minimum release at four hours. EP showed the highest fluoride release after varnish application. In our study, Enamel Pro showed the highest fluoride release at four hours following its application, which was in agreement with the results of Ritwik et al, [13] (2012). In the study by Cochrane et al, [11] (2014) calcium ,non organi phosphate and fluoride release of MI varnish, Enamel Pro, Clean Pro White and Durapha, Bi fluorid5 were compared. Enamel Pro showed the highest fluoride release in the first hour and MI and then Enamel Pro had the highest fluoride release at four hours.

Evidence shows that the fastest fluoride release occurs in the first seven hours and then within the first week following application. In our study, Enamel Pro had the highest fluoride release at four hours and the lowest at 72 hours, showing a descending trend of release while Preventa had the lowest release at four hours and the highest at 72 hours, indicating an ascending trend of fluoride release. This difference between the two varnishes is probably attributed to the carriers used in their composition since the carrier can affect the fluoride release potential of products [13]. Seppa et al, [16] (1995) in their study on efficacy of fluoride varnish and phosphate fluoride gel indicated that in proximal caries Duraphat (sodium fluoride varnish) was as effective as Nupro (acidulated phosphate fluoride). Shen and Autio-Gold [16] (2002) also showed that the fluoride content of Duraphat was more uniform than that of Cavity Shield.

Fluoride products should be administered in children for prevention of caries. In our study, V-Varnish has more fluoride release than Enamel pro up to 24 hour . however, whether or not this higher release results in higher uptake of fluoride by the enamel needs further investigations. Thus, future studies are recommended to quantify the fluoride uptake by the enamel after the application of different fluoride varnishes with different fluoride release potentials. Moreover, in vivo studies are required to cast a final judgment regarding this topic.

CONCLUSION

The amount of fluoride release during the time, both in Enamel pro and V-Varnish had significant difference and at one hour up to 24 hour V-Varnish had more fluoride release than Enamel pro and at 72 hour the amount of fluoride release in Enamel pro was more than V-Varnish.

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