Original Article

In vitro evaluation of the effect of fluoride gel and varnish on the demineralization resistance of enamel

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Abstract

***Background and Aim*:** Fluoride has an important role in the prevention of caries. This study assessed the efficacy of three types of topical fluoride: fluoride varnish and two brands of APF gel in protecting enamel from demineralization in vitro.

***Materials and Methods*:** Sixty human caries-free premolars where randomly assigned to four groups of 15 . The control group was washed with deionized/distilled water. Teeth in group A were weekly treated by 1.23% APF gel (Kimia Co, Iran) for 2 minutes, whereas teeth in group B were weekly treated with 1.23% APF gel for 60 seconds and those in group C were treated with 2.26% Durashield. Specimens were then placed in demineralization (pH= 4.3) and remineralization (pH= 7) cycles for 6 and 17 hours, respectively. This pH- cycle was repeated for 3 weeks. The teeth were sectioned buccolingually and evaluated under a polarized light microscope. Then the depth of each lesion was measured from the deepest demineralization point of the lesion. Data were analyzed using Kruskal-Wallis and Dunn test for pairwise comparison.

***Results*:** The control group had the deepest lesions (mean depth, 140±37micrometer). Group C had the shallowest lesions (mean depth, 60±37 micrometer) with a 75.3% reduction compared with control. However, there was no significant difference in the depth of demineralization defects among all fluoride treated groups, while the difference between fluoride and control groups were significant.

***Conclusion*:** Treatment of the enamel of permanent teeth with various topical fluo Ride gels significantly inhibited demineralization, but there was no significant difference between varnish or gel application.

***Key Words*:** Fluoride varnishes - APF gel – Demineralization - Enamel

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Introduction

Tooth decay is the most common chronic disease in childhood. Topical fluoride therapy has shown positive results in prevention of tooth decay (1). Topical fluorides are presented in different types such as tooth pastes, fluoride containing mouth washes, gels, foams and fluoride varnishes. Fluoride has two main mechanisms; namely, preventing demineralization of the normal enamel and improving enamel remineralization through incorporation of fluoride in enamel composition(2). Application of acidulated phosphate fluoride (APF) gel is the most common professional fluoride therapy method in many countries. Its caries preventive effect has been mentioned in several studies, (2, 3), although fluoride swallowing is considered one of the disadvantages of this method in young children(4). In the last two

decades, application of fluoride varnish has been very much popular, due to its advantages. Ease of manipulation, safety and simplicity of application procedural steps are considered as the reasons for its widespread acceptance. (3, 4). Fluoride varnish requires less treatment time in comparison with fluoride gel, which needs tray and suction for its application. The exposure time between fluoride and the tooth surface increases when fluoride varnishes are used due to its adhesion to the tooth surface. (5). The concentration of fluoride in fluoride varnishes such as Dura shield (22600 ppm) is two-fold that of APF gels (12300 ppm). It has been mentioned that the concentration and exposure time of topical fluorides affect the properties of fluoride reaction on the tooth surface (1). Few clinical evaluations have compared the effect of fluoride gel and varnish on the prevention of dental caries and their results are incongruent (3, 6, 7). Seppa et al. in a three-year clinical trial reported that fluoride gel and varnish had equal effects on children's tooth decay (3); on the contrary, in a two-year study on children, Tewari et al. showed that fluoride varnish had a stronger effect on preventing tooth decay (7).

Only one laboratory study showed that weekly use of fluoride varnish has a similar effect to daily usage of Klarigel-N fluoride gel regarding increase in resistance against dentinal caries of the root surface (8). There has been no study evaluating the effect of APF gel and sodium fluoride varnish on the enamel's demineralization resistance. Based on the fact that various Iranian topical fluoride gel preparations are introduced with a great difference between the prices of domestic and foreign productions, comparing the difference between the effects of these products is an important concern for Iranian dentists. Although clinical trials are considered as gold standard, these studies are expensive, time-consuming and controlling their confounding factors is also difficult; therefore, use of laboratory studies is a valuable and efficient tool for evaluation of the therapeutic and caries-prevention abilities of fluoride (9). The aim of this study was to compare the Iranian (Kimia) APF gel, the foreign (Sultan) APF gel and the sodium fluoride varnish (Sultan) regarding the permanent enamel's demineralization resistance in a pH-cycle.

Materials and Methods

In this experimental laboratory study, 60 sound premolar teeth which were extracted for orthodontic reasons within the last six months were included and were inspected to be without any cracks, fractures or restorations. Samples were disinfected with 13% sodium hypochlorite solution for 24 hours and stored in normal saline at room temperature (10).

First, the teeth were polished with pumice powder and distilled water for 10 seconds. Then a 4×2 mm window was placed horizontally by paper sticker on the buccal surface of the tooth and the remainder of the tooth surface was covered by nail polish (MY) in two stages. The first layer of nail polish was applied and after three hours the teeth were covered by the other layer. After 24 hours, the label was removed. The remainder of the label was removed by alcohol and in order to be sure of the cleanness of the window, this surface was controlled by a stereomicroscope (11).

The prepared teeth were randomly divided into four groups of 15 and fluoride therapy was performed for three weeks according to the following program.

Control group: The teeth were contacted with deionized water once a week for two minutes by a micro brush.

Group A: Teeth were exposed to 1.23% APF gel (Kimia Chemicals, Tehran, Iran) for 4 minutes with a micro brush ,once a week .

Group B: Teeth were exposed to fluoride gel (Sultan Chemist, Englewood NJ USA, Topex) containing 1.23% APF and a pH of 3.5 once a week for one minute by a micro brush according to the manufacturer's guide.

Group C: Teeth were exposed to 5% sodium fluoride containing Dura shield fluoride varnish (Sultan Chemist, Englewood NJ USA, Topex), once a week, based on the manufacturer's guide.
After finishing all the above mentioned stages, the samples were rinsed with deionized water and entered into the pH-cycle. After 24 hours the varnish in group C was removed by a blade (8). All the samples were placed in a separate container for each group containing 2.2 mM CaCl2, 50 M CH3COOH and 2.2 mM KH2PO4 demineralization solution for 6 hours after fluoride therapy. After 6 hours, the samples were brought out and rinsed with deionized water for 20 seconds to remove the demineralization solution, then placed in CaCl2 0.9 mM KH2Po4 150 mMKCl 1.5 mM remineralization solution for 17 hours. The samples were then brought out and rinsed with deionized water and were subsequently entered into the next cycle. The volume of each solution for each sample was 10 ml. All these stages were carried out in an incubator (Shimifan, Iran) at 37ºC temperature. At the beginning of each week after the remineralization step, the samples were rinsed with deionized water and the solutions were changed before the second round fluoride therapy and the pH of the solutions were controlled (8).
After the testing period, all the samples were cut into two equal parts parallel to the longitudinal axes of the teeth from the middle of the window by a Discoplan-TS (Struers) cutting and grinding two-action machine. Then the samples were polished moistly with carborundum powder (400 and 800) and stabilized on the slide via the polished side by Eukitt adhesive (synthetic thermoplastic resin). Three hours after stabilization, the thickness of the samples were set at 100±30 micrometer, using the grinding machine. Sections were observed by polarized-light microscope (Olympus BH-2) with 10x ocular and 5x optical magnifications and using distilled water as the medium agent. In every sample, the depth of the lesion, from the deepest point to the surface of the lesion was measured (8).
According to the recorded table for each microscope, each degree of the graded lens with 50x magnification showed 20 micrometers of the sample. In order to measure the depth of the lesion in the samples, the number of the slides were covered by stickers and two educated observers who were not informed about the classification of the samples carried out the measurement.
The raw data were entered to SPSS for windows 15. The percentage of decrease in the lesion depth was measured for the test groups compared with control. For evaluation of the distribution of the data, Kruskal Wallis test with a confidence interval of 0.05 was used and Dunn test was utilized for pairwise comparison of the groups.

Results

The study was performed on 60 premolar teeth with the necessary qualifications. The data regarding measurement of the lesion depth in the evaluated groups were recorded in SPSS software and the mean and standard deviation and the percentage of decrease was measured for each group (Figure 1) (Table 1).

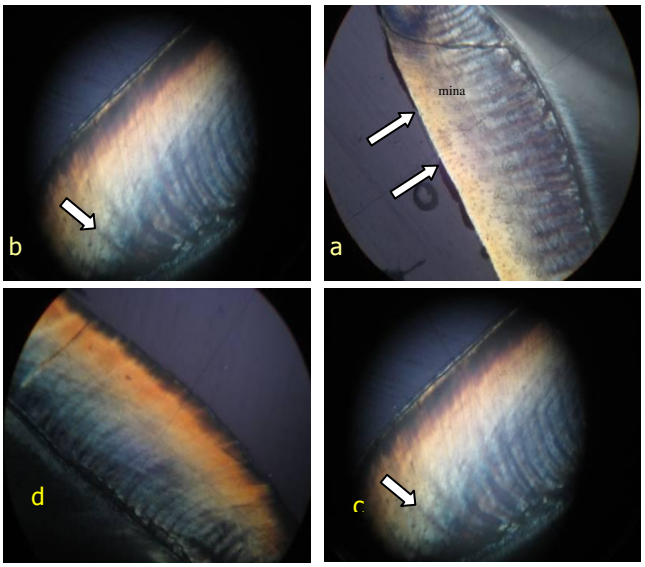

Fig. 1: A. Microscopic appearance of the samples without decay in the 5% NaF fluoride varnish group. The arrows show the boundaries of the window.

arrows show the boundaries of the window. B. Microscopic appearance of the control group with decay. The arrow shows the depth of the demineralization.C. Microscopic appearance of the 1.23% APF Iranian gel (Kimia). The arrow shows the depth of the demineralization.

B. Microscopic appearance of the control group with decay. The arrow shows the depth of the demineralization.

C. Microscopic appearance of the 1.23% APF Iranian gel (Kimia). The arrow shows the depth of the demineralization.

As mentioned in Table 1, the control group had the deepest lesions (range, 100-200; mean, 140 micrometer). In group C (range, 0-120 micrometer; mean, 45.33), the percentage of lesion depth decrease compared to the control group was 67.7%. In group A (range, 0-100 micrometer; mean, 34.66 micrometer), the percentage of lesion depth decrease was estimated as 75.3% in comparison with control. According to table 1, the lowest mean for the lesion depth was related to group C and the highest value belonged to the control group.

In the next step, in order to evaluate the data distribution and analysis of the data for each test group, Kruskal Wallis test was used and P <0.05 was considered as statistically significant. There was no significant difference observed statistically between the means of the groups (Table 2). Measurement of the depth of the lesions and pairwise comparison of the groups was performed by Dunn test. As demonstrated in Table 2, comparison of the depth of the lesions in test groups (Sultan fluoride gel, Kimia fluoride gel and fluoride varnish) showed no significant difference statistically, but the difference between the control group and the test groups was statistically significant (p<0.05).

Table 1: The Mean and Decrease Percentage of Demineralization Depth in the Studied Groups Using Polarized Light Microscope

Group	Number	Minimum	Maximum	Mean	Standard Deviation	Decrease Percentage
Fluoride Varnish	15	0	100	34.66	38	75.3
Kimia Fluoride Gel	15	0	200	60	59	57.2
Sultan Fluoride Gel	15	0	120	45.33	41	67.7
Control	15	100	200	140	37	0

Table 2: Pairwise Comparison of the Groups Using Dunn Test

Group	Fluoride Varnish	Kimia Fluoride Gel	Sultan Fluoride Gel	Control
Fluoride Varnish		-	-	*
Kimia Fluoride Gel			-	*
Sultan Fluoride Gel				*
Control				

***:Statistically Significant**

-:Statistically Not Significant

Discussion

Topical fluorides showed positive results regarding tooth decay prevention (1). Fluoride varnish has a neutral pH and , is taken into consideration by pediatric dentistry due to its ease of application and safety. Application of fluoride varnish takes less time in comparison to fluoride gel.

Fluoride varnish hardens after exposure to saliva; consequently, sticks to the tooth surfaces, thereby increasing the contact between fluoride and tooth.

Varnish contains a higher concentration of fluoride (two-fold that of an APF gel) (3, 4). APF gel with its acidic pH, has been the most common effective topical fluoride regimen used for caries prevention for years in many countries (2, 3). Although the efficiency of professional topical fluorides in the prevention of caries has been proved, the type with the greatest effect has not been identified. Limited clinical studies have focused on the effect of varnish and fluoride gel on preventing tooth decay. These studies have reported different results (2). APF fluoride gel has been produced for professional use in Iran, too. Based on the difference between the price of foreign and Iranian APF gels, information regarding their differences may help dentists choose the appropriate product.

The present study evaluated the role of 1.23% APF gel (Kimia, Iran), 1.23% APF gel (Sultan, foreign) and 2.26% fluoride varnish (Sultan, Durashield) in preventing enamel demineralization in a pH cycle model. The results of this study showed that fluoride varnish and Sultan and Kimia fluoride gels may prevent enamel demineralization in an acidic model, but none of these products were able to prevent demineralization completely. Fluoride varnish, Sultan fluoride gel and Kimia fluoride gel were able to decrease simulated carious lesion depth to 75.3%, 67.7% and 57.2%, respectively. Although fluoride varnish was able to decrease the caries depth more than the other products, there was no significant difference between the three products regarding resistance to demineralization. Fluoride varnish had almost two-fold higher concentration in comparison to that of gel and it also had a longer contact duration with the teeth, but its protective effect against demineralization was similar to that ot APF gel.

After using topical fluoride with a high concentration, calcium fluoride (CaF2) is the main precipitating product on the enamel surface and the subsurface enamel carious lesions. Products with a low concentration of fluoride tend to precipitate fluoroapatite [$Ca_{10}(Po_4)_6F_2$]. Although fluoroapatite bonds to the crystalline structure of the enamel, most of the precipitated calcium fluoride on the enamel surface disappears in contact with alkaline solutions. In demineralization circumstance and when a decrease in environmental pH ensues and phosphate ions are present, the fluoride ion released from calcium fluoride is able to precipitate as fluoroapatite in the enamel structure (2, 5).

Studies have shown that calcium fluoride is the main product of the reaction between APF and enamel surface. As a result of acidic pH (3.5) and increase in the concentration of phosphate in the reaction environment, more fluorohydroxyapatite is produced (2, 5, 12). Therefore, it may be concluded that a neutral fluoride varnish with a higher concentration of fluoride may be similar to acidic APF gel with a lower concentration regarding prevention of demineralization.

Murakami et al. in accordance with the present study showed that fluoride varnish and APF gel decrease the loss of minerals and prevent erosive lesions in the laboratory environment similarly. Application of acidic APF gel seems to precipitate a higher amount of calcium fluoride on the surface of the enamel in comparison with neutral gel; therefore, higher protection of the enamel is possible even with lower concentrations of fluoride (13). In the literature, the effect of different concentrations of fluoride varnish have been assessed on demineralization. It has been mentioned that the efficiency of varnish has no association with its concentration, but is related with the times it has been applied (2).

Hong et al. have evaluated the effect of Karigel-N gel (5000 ppm) and sodium fluoride varnish (22600 ppm) in preventing dentin and root caries *in vitro* in two separate studies. The results showed that weekly use of fluoride varnish resulted in a higher prevention of dentin and root demineralization in comparison with weekly use

of Karigel-N gel (8, 14). The present study evaluated the effect of flouride varnish and APF gel on enamel. The low pH of APF gel led to etching of enamel and an increase in surface roughness. This etching increases the entrance of fluoride into enamel. Hong et al.'s studies assessed the effect of fluoride varnish and neutral Karigel-N gel on the surface of dentin and root cement. Ganss et al. mentioned that difference in the study design and type of dental substrates may affect the results (13, 15).

The results of the present study showed that there was no significant difference statistically between two fluoride gels regarding the percentage of lesion depth decrease. This result may support Iranian products and may help dentists in choosing the appropriate substance. In order to extrapolate the results of this study to clinical conditions, there are other considerations such as gel consistency, penetration into the interdental spaces and acceptance by the patient which is influenced by its taste and ease of application. Kimia APF gel is noticeably more consistent than Sultan APF gel with a hotter taste. Lately, a new thixotropic gel has been introduced with increased capability to penetrate into the interdental spaces (2); therefore, more studies are necessary to evaluate the effect of this aspect in Kimia and Sultan APF gels.

In order to stimulate caries, there are many different models. In our study, we used the pH-cycle method to design an environment resembling the oral cavity. This model simultaneously measures the outcome of demineralization inhibition and remineralization enhancement and includes de- and re-mineralization solutions. The time necessary for demineralization was 6 hours a day and 17 hours was needed for remineralization which is exactly similar to the time when oral pH is acidic in 24 hours. This demineralization-remineralization cycle resembles the oral environment when food enters the mouth.

Before being transferred, the samples were rinsed with deionized water for 30 seconds in order to prevent the influence of the solutions on each other (8, 14, 16, 17).

In this study, there were some limitations, for instance; the teeth were all young and recently extracted for orthodontic purposes and were gathered from different dental clinics in Tehran. Because of the difference in the water fluoride, there may be the possibility of difference in the fluoride content of the teeth. Another limitation was that the demineralization and remineralization solutions were changed once a week. Although the samples were rinsed with deionized distilled water twice a day, the release of fluoride ion from the gel and varnish into the solutions might have led to different contamination levels.

In this study, for measurement of lesion depth a graded lens of polarized light microscope was used. Two educated observers who had no information about the study groups evaluated each section leading to decrease in the probability of measurement error.

Polarized light microscopy (PLM) is a standard method in demineralization/remineralization studies of the teeth.

PLM may give an exact measurement of the lesion depth and its extension, but gives no additional information such as change in the mineral density. Using PLM, a semi quantitative evaluation may be performed. This evaluation obtains valuable information regarding the reciprocal effect of the substance on the enamel, the dentin and the relatively demineralized hard tissue. Further studies to evaluate subsurface lesions by microradiography or cross sectional microhardness testing is also suggested (20).

Conclusions

1-Iranian and foreign APF gel and sodium fluoride varnish were significantly different in comparison with the control group regarding prevention of demineralization.

2-Although fluoride varnish decreases the lesion depth more than the APF gels do, there were no

significant differences between fluoride varnish and Iranian and foreign APF gels.

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