Evaluation of The Effect of Fluoride Gel and Varnish on The Demineralization Resistance of Enamel: An in Vitro

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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, APF gel (sultan), APF gel (Kimia) in protecting the enamel from demineralization in an in vitro environment.

Materials and Methods: Sixty human caries-free premolars where randomly assigned into four groups of 15 specimens. The control group was washed with deionized/distilled water. Weekly gel (Kimia) was treated with APF gel (1/23%) for 2 minutes weekly, weekly gel (Sultan) was treated with APF gel (1/23%) for 60 seconds weekly, weekly varnish fluoride was treated with Durashield (2/26%). Specimens were then placed in a cycle of demineralization (pH= 4.3) for 6 hours and remineralization (pH= 7) for 17 hours. This pH-cycle was repeated for 3 weeks (21days). The teeth were sectioned buccolingually and evaluated under polarized light microscope. Then the depth of each lesion was measured from the deepest demineralization point of the lesion. The data were analyzed using Kruskal-Wallis and Dunn test for pairwise comparison.

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

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

Key Words: Fluoride varnishes, APF gel, Demineralization, Enamel

Introduction

Tooth decay is the most common chronic disease of childhood. Topical fluoride therapy has shown positive results in the 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 the remineralization of the enamel using fluoride in the dental enamel [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 swallowing fluoride is
one of the disadvantages of this method [4]. In the last two decades, appliance of fluoride varnish due to its advantages has been very much popular. The reasons of its acceptance are mainly, the easy application, safety and easy work stages [3, 4]. Both fluoride varnish and fluoride gel are applied by tray and suction, but fluoride varnish needs less time in comparison to fluoride gel. Because of adhesion of the varnish to the tooth surface, the exposure time between fluoride and the tooth surface increases [5]. The concentration of fluoride in fluoride varnishes such as Dura shield (22600 ppm) is twice-fold 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 tooth caries and their results are incongruent [3, 6, 7]. Seppa et al. in a three-year clinical trial reported that gel and varnish had an equal effect on children’s tooth decay [3]; on the contrary, Tewari et al. in a two-year study on children showed that fluoride varnish had a higher effect on suppressing 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 to dentinal caries of the root surface [8]. There has been no study evaluating the effect of APF gel and NaF varnish on the the enamel’s demineralization resistance. Based on the fact that there has been many Iranian topical fluoride gel productions and there is great difference between the prices of internal and external productions, knowing the difference between the effects of these products is an important matter for Iranian dentists. Although clinical trials are the gold standard, these studies are expensive, time-consuming and controlling the confounding factors is also difficult; therefore, use of laboratory studies is a valuable and efficient tool for evaluation of the therapeutic and anti decay abilities of fluoride [9]. The aim of this study was to compare the Iranian (Kimia) APF gel, the foreign (Sultan) APF gel and the NaF 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 with no cracks, fractures and restorations which were extracted for orthodontic reason in the last six months were gathered and after disinfection with 13% hypochlorite for 24 hours, were placed in normal saline and maintained in 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 second layer of nail polish. After 24 hours, the label was removed. The remainder of the label was removed by alcohol and in order to be sure of the cleanliness of the window, this surface was controlled by a stereomicroscope [11]. The prepared teeth were randomly divided into four groups of 15 teeth and fluoride therapy was performed for three weeks according to the following program.

Control group: Once a week for two minutes by a micro brush, the teeth were contacted with deionized water.

Foreign fluoride gel group: Once a week the teeth were exposed to fluoride gel (Sultan Chemist, Englewood NJ USA, Topex) containing 1.23% APF and a PH of 3.5 for one minute by a micro brush according to the manufacturer’s guide.

Iranian fluoride gel group: Once a week the teeth were exposed to 1.23% APF Iranian fluoride gel (Kimia) for 4 minutes with a micro brush.

Fluoride varnish group: Once a week the teeth were exposed to 5% sodium fluoride containing...
Dura shield fluoride varnish (Sultan Chemist, Englewood NJ USA, Topex) based on the manufacturer’s guide.

After finishing all the above mentioned stages, the samples were rinsed with deionized water and were entered into the PH-cycle. In order to equalize the lost gel and varnish, after 24 hours the varnish 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, rinsed with deionized water for 20 seconds to take away the demineralization solution, then placed in CaCl2 0.9 mM KH2PO4 150 mM KCl 1.5 mM remineralization solution for 17 hours, then the samples were brought out and rinsed with deionized water and were subsequently entered into the next cycle. The volume of each of the solutions for each sample was 10 ml. All these stages were carried out in an incubator (Shimifan, Iran) in 37°C temperature.

At the beginning of each week after the remineralization step, the samples were rinsed with deionized water and before the second fluoride therapy the solutions were changed 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 axis 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 mostly 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, using the grinding machine, the thickness of the samples were set at 100±30 micrometer. The sections were observed by distilled water as the medium and polarized light microscope (Olympus BH-2) eye magnification ×10 and lens magnification ×5. 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 ×50 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 of the classification of the samples carried out the measurement. The raw data were entered to SPSS for windows 15. The percentage of the lesion depth decrease was measured for the test group compared to the control group. 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 as the control, APF gel (Kimia), APF gel (Sultan) and the NaF varnish groups. 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 the Sultan fluoride gel group (range, 0-120 micrometer; mean, 45.33), the percentage of lesion depth decrease compared to the control group was 67.7%. In the fluoride varnish group (range, 0-100 micrometer; mean, 34.66 micrometer), the percentage of lesion depth decrease of this group in comparison to the control group was estimated as 75.3%. In the Kimia fluoride gel group (range, 0-200 micrometer; mean, 60 µm), the percentage of lesion depth decrease was estimated as 57.2%. The lowest mean depth of the lesion was for the fluoride varnish group and the highest mean depth of the lesion belonged to the control group (Table 1).

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 lower than 0.05 was statistically significant. The results of this study showed that 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. The difference between the control group and the test groups was statistically significant (p<0.05).

Discussion
Topical fluorides showed positive results regarding tooth decay prevention [1]. Fluoride varnish has a neutral PH and due to the easy application, safety and easy work stages, is taken into consideration by pediatric dentistry. Application of fluoride varnish takes less time in comparison to fluoride gel.

Varnish fluoride hardens after exposure to saliva; consequently, sticking to the teeth and as a result contact of fluoride with the teeth increases.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Decrease Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoride Varnish</td>
<td>15</td>
<td>0</td>
<td>100</td>
<td>34.66</td>
<td>38</td>
<td>75.3</td>
</tr>
<tr>
<td>Kimia Fluoride Gel</td>
<td>15</td>
<td>0</td>
<td>200</td>
<td>60</td>
<td>59</td>
<td>57.2</td>
</tr>
<tr>
<td>Sultan Fluoride Gel</td>
<td>15</td>
<td>100</td>
<td>120</td>
<td>45.33</td>
<td>41</td>
<td>67.7</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>0</td>
<td>100</td>
<td>140</td>
<td>37</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 2: Pairwise Comparison of the Groups Using Dunn Test

<table>
<thead>
<tr>
<th>Group</th>
<th>Fluoride Varnish</th>
<th>Kimia Fluoride Gel</th>
<th>Sultan Fluoride Gel</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoride Varnish</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>Kimia Fluoride Gel</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>Sultan Fluoride Gel</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*:Statistically Significant
-:Statistically Not Significant
Varnish contains a higher concentration of fluoride (two-fold APF gel) for the prevention of tooth decay [3, 4]. APF gel–acidic PH–has been the most common effective topical fluoride used for the prevention of tooth decay 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. Till now 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 from years before in Iran. 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 75.3%, 67.7% and 57.2% of the caries depth, 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 gel and it also had a longer contact duration with the teeth, but its protective effect against demineralization was similar to 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 of the enamel decay lesion. Products with a low concentration of fluoride have the predilection to precipitate as fluoroapatite [Ca10(PO4)6F2]. Although fluoroapatite bonds to the crystal structure of the enamel, most of the precipitated calcium fluoride on the enamel surface disappears in contact with alkaline solutions. In demineralization circumstances, decrease in environmental PH and presence of phosphate ion, 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 the 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 erosion 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 to 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 NaF varnish (22600 ppm) in preventing dentin and root decay in the laboratory environment 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 to the weekly use of Karigel-N gel [8, 14]. The present study evaluated the effect of fluoride varnish and APF gel on the enamel. The low PH of APF gel
led to etching of the enamel and increase in the surface roughness. This etching increases the entranece of fluoride into the enamel. Two of 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 the 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 Iranian and foreign 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 generalize the results of this study to clinic, there are other considerations such as gel consistency, penetration into the interdental spaces and acceptance by the patient due to its taste and easy appliance. Kimia APF gel is noticeably more consistent than Sultan APF gel with a hotter taste. Lately, a new thixotropic gel has been introduced; primarily it has a gel consistency, but after force entrance changes to solution consistency in order 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 decay, 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 true results of suppression of demineralization and increase of remineralization and includes demineralization and remineralization 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 that 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 limitsations; the teeth were all young teeth–extracted as a result of orthodontic treatment–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 may 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. We suggest another study to evaluate the subsurface lesion by microangiography or cross sectional microhardness [20].

Conclusions
1-Iranian and foreign APF gel and NaF varnish were significantly different in comparison to the control group regarding prevention of demineralization.

2-Although fluoride varnish decreases the lesion depth more than the APF gels, there were no significant differences between fluoride varnish and Iranian and foreign APF gels.
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