The Effect of Silver Diamine Fluoride and Fluoride Varnish on Roughness of Primary Teeth Enamel (An In Vitro Study)

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**ABSTRACT**

**Aims:** The study aimed to evaluate and compare the effect of fluoride varnish and silver diamine fluoride solution on surface roughness of enamel of primary teeth in vitro study. Materials and methods: A total of (150) primary anterior teeth were used. Enamel blocks were prepared and divided into three groups: Fluoride varnish group (n=50), silver diamine fluoride group (n=50), and the control group of deionized water (n=50). PH cycle completed at one day and repeated for 10 days. Specimens were kept in demineralizing solution for 3 hours and remineralizing solution for 20 hours. All specimens were washed in deionized water between solutions and placed in artificial saliva for 30 minutes at the end of the demineralization and remineralization process. Surface roughness was measured before and after PH cycle. **Results:** There was no significant difference between groups before PH cycle while at the end of PH cycle, groups showed highly significant difference at p ≤ 0.01. Also, silver diamine fluoride had the lowest mean value for surface roughness followed by varnish group while the maximum increase in surface roughness belonged to the control group of deionized water. **Conclusions:** Silver diamine fluoride solution was a significantly better than fluoride varnish in preserving surface characteristics of the enamel of primary dentition.

**Key words:** Enamel demineralization, Silver diamine fluoride, surface roughness, primary dentition.

DOI: 10.33899/rden.2020.127479.1040 ©2020, College of Dentistry, University of Mosul
INTRODUCTION

Dental caries is an international public health challenge, particularly among young children\textsuperscript{(1)}. Primary teeth are susceptible to dental caries. Caries in primary teeth may hamper eruption and tooth structure formation of permanent teeth and for sure affects the oral health status of children\textsuperscript{(2)}.

Dental caries originates from the Latin word \textit{(caries)} which means decay, this term was used to describe teeth having holes. It is one of the most common and oldest diseases found in the human. Caries is a chronic infectious transmissible disease caused by specific adherent bacteria, particularly Streptococcus mutans that produces acid by the metabolism of sugar and demineralizes tooth structure over time\textsuperscript{(3)}. Importantly, primary teeth have greater permeability, lower bonding strength to dental adhesive materials, and lower microhardness in comparison to permanent teeth\textsuperscript{(4)}. The lower dimension of the hydroxyapatite lattice, when compared to permanent dentition, plays an important role in lowering the acid resistance of deciduous teeth\textsuperscript{(5)}. Early childhood caries cause dental destruction, pain, and place the permanent dentition at risk for developing dental caries. Also, It can negatively affect the quality of life of children and their caregivers\textsuperscript{(6)}.

Fluoride is widely used as a remineralizing agent and based on a systematic review, 5% NaF varnish was more effective in remineralizing early enamel caries\textsuperscript{(7)}. As a result of fluoride side effects\textsuperscript{(8)} and NaF varnish shortcomings\textsuperscript{(9)}, looking for alternatives could be valuable, metal ions are alternatives for preventing dental caries\textsuperscript{(10)}.

Interest in SDF in recent several studies proved its effectiveness in preventing and arresting caries in the primary dentition and first permanent molar in children\textsuperscript{(11-12)}. Silver Diamine Fluoride (SDF) is considered an effective agent for the prevention and control of dental caries in primary teeth\textsuperscript{(13)}. SDF has been suggested for difficult-to-treat caries lesions and patients at high risk of developing caries including those with medical or behavioral complications, those who require multiple treatment visits, or those without access to dental care\textsuperscript{(14,15)}.

MATERIALS AND METHODS

Criteria of sample collection:
Intact upper and lower primary anterior teeth were collected, being caries free, having no fillings, no developmental anomalies, no enamel hypoplasia, no cracks, wears or fractures. Also enamel surface should be unaffected by a chemical agent as a bleaching agent or acid etching.

Teeth Samples Preparation:
The extracted teeth were cleaned, washed with deionized water and kept in thymol solution 0.1\% to keep the samples wet, and to avoid the growth of bacteria and fungi on teeth surfaces\textsuperscript{(16,17,18)}. The extracted primary teeth had been examined under stereomicroscope (UNION /JAPAN). Only the intact teeth had
been included in the study and about fifteen teeth had been excluded. Teeth had been cleaned, polished with non-fluoridated pumice and rubber cups, the remaining roots had been cut 2mm below the level of cement enamel junction using a straight diamond bur with copious water irrigation to avoid harming the crowns. All teeth are thoroughly washed with deionized water and kept in a 0.1 % thymol solution until being mounted in a chemical cured resin in the plastic rings. Plastic rings had been cut and prepared in such a way that the upper and lower sides had been flat and parallel to each other (16mm diameter ×14mm depth).

Each ring will have one primary tooth that is fixed on the top surface of the ring in the center with the labial surface of the tooth exposed so that enamel block of 4x4mm window was obtained (19). After setting of cold cure acrylic, the rings with the exposed buccal surfaces of teeth were polished with a fin grit silicon carbide paper (600-, 800-, and 2400-grit) respectively. Lastly, all samples were washed with deionized water and kept till the starting of pH cycle.

Materials:
The materials used in this study are:
1- Fluoride Varnish / Ftor-Lux (sodium fluoride 1%, Calcium fluoride 4%, Aminofluoride 0.5%) / Techno Dent / Russia.

2- Silver Di Amine Fluoride solution / CARIESSTOP 30%/ biodinamica / Brasil / PH 8.5.

The Experimental Design of the Study:
The total number of teeth samples in the main study was (150) samples. Teeth Samples were randomly divided into three main groups, each one contains (50) teeth samples as follows:

Group 1 (fluoride Varnish): The teeth were exposed to fluoride varnish for 24 hrs and stored in deionized water, then the layer of varnish was removed with the scalpel blade and cotton soaked with acetone. Being careful not to scratch the surfaces, samples washed with deionized water for 1 min then introduced to pH cycle (19).

Group 2 (Silver diamine solution): the teeth surfaces were exposed to silver diamine fluoride solution with a small brush for 2 min then washed with deionized water for 30 sec and lightly dried with absorbent papers, then introduced to PH cycle (19).

Group 3 (Control): no treatment was applied, only washed with deionized water.

Solutions preparation:
Chemical materials were imported from College of Sciences (Department of Chemistry, College of Dentistry (Department of Basic Dental Sciences) and the Main laboratory of the University of Mosul after getting the formal transaction to deal and barrow chemical materials. According to the laboratory general
safety rules, chemical materials had been handled after weighting it by accurate digital balance, then mixing it in one liter of deionized water until it completely dissolved. PH meter had been used to check the PH of the prepared solutions. Preparation of chemical solution was done at the College of Dentistry \ Department of Basic Sciences at the chemistry laboratory.

1. Demineralization solution: The demineralizing solution consist of CaCl$_2$ (2.2 mM), NaH$_2$PO$_4$ (2.2 mM), and acetic acid (0.05 M), pH of 4.5, adjusted with KOH (1M), 15 ml/tooth$^{(20)}$.

2. Remineralization solution: The remineralizing solution consist of CaCl$_2$ (1.5 mM), NaHPO$_4$ (0.9 mM), KCl (0.15 mM), pH of 7.0, 15 mL/tooth$^{(20)}$.

3. Artificial saliva: Components of artificial saliva are NaCl 0.40, KCl0.40, CaCl$_2$ .2H$_2$O 0.79, NaH$_2$ PO$_4$ .2H$_2$O 0.78, NaS9.H$_2$O 0.005,CO(NH$_2$)2 Urea 0.1, in 1000 ml Distilled water, pH of 7 ( concentration G \ L)$^{(21)}$.

The unclear, cloudy liquid of remineralizing solution and artificial saliva had been distillated using filter papers, after that when minerals had been completely stabilized the PH was measured to be sure no changes of PH readings will occur days later.

PH-cycling:

Teeth were submitted to the formation of artificial caries by PH cycling. keeping the teeth in demineralizing solution PH of 4.5 for 3 hours and in remineralizing solution PH of 7.0 for 20 hours. All the teeth were briefly washed in deionized water between solutions and placed in artificial saliva for 30 minutes at the end of the demineralization process and for 30 minutes at the end of the remineralization process.

The duration of each cycle was one day (24 hours) and the teeth were subjected to a total of 10 cycles. The demineralizing-remineralizing solutions were changed daily, and the artificial saliva was changed at every treatment$^{(22, 23)}$.

Surface Roughness Test:

The surface roughness of the enamel surface samples measured by using a profile meter (Profile Projector/ MITYOTO/JAPAN) as shown in (Figures 2, and 3) with the magnification of 50X.
Experimental Design of the Study (150) teeth samples

Group 1: (50) teeth samples. Fluoride varnish (futor lux)

Group 2: (50) teeth samples. Silver diamine fluoride

Group 3: (50) teeth samples. (Control) de ionized water.

**Figure (1):** Experimental Design of the Study

**Figure (2):** Roughness profilometer.

**Figure (3):** Enamel surface samples measured by using a Mityoto J-250/profile projector
The test was conducted at a Technical Institute / Mosul University. Surface roughness was characterized by the arithmetical average of the surface showed maximum and minimum lines drawn at the highest peak and lowest valley found within a central line along the area\(^{(24)}\).

We use maximum peak valley height (Ry) way of measurement as shown in (Figure 4) which includes: A section of standard length is sampled from the mean line on the roughness chart. The distance between the Maximum peak (Rp) and valley (Rv) of the sampled line is measured in the Y direction. The value is expressed in micrometer (μm)\(^{(25)}\).

![Figure 4: Maximum peak valley height (Ry)](Image)

The cutoff value or reference length was adjusted to act at 0.8 mm. Three measurements of surface roughness were performed for each sample\(^{(26,27)}\) and the average of these readings was used for the statistical analysis.

**RESULTS**

Regarding the Table (1) the descriptive statistics of the results of surface roughness test which includes the means, standard deviations and numbers of samples of all study groups before and after PH cycle of initiating early enamel caries was shown there was the minimum increase in surface roughness of silver diamine fluoride group followed by fluoride varnish group and the maximum increase in surface roughness belonged to the control group of deionized water.
Table (1): Descriptive statistics including the means, standard deviations and numbers of samples among tested groups before and after PH cycle.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Before PH Cycle</th>
<th>After PH Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoride Varnish</td>
<td>Mean</td>
<td>0.6470</td>
<td>0.7668</td>
</tr>
<tr>
<td></td>
<td>Std. deviation</td>
<td>0.12658</td>
<td>0.12445</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Silver Di Amine Fluoride</td>
<td>Mean</td>
<td>0.6222</td>
<td>0.6944</td>
</tr>
<tr>
<td></td>
<td>Std. deviation</td>
<td>0.13006</td>
<td>0.11053</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>De-ionized Water</td>
<td>Mean</td>
<td>0.6634</td>
<td>0.9692</td>
</tr>
<tr>
<td></td>
<td>Std. deviation</td>
<td>0.14011</td>
<td>0.22421</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Table (2) ANOVA test explaining the significant difference between tested groups before and after pH cycle while after the addition of test materials and introduction into PH cycle, there was highly significant difference among groups.

Table (2): ANOVA test demonstrating the difference among test groups before and after PH cycle, and explaining that there was no significant difference among groups before PH cycle.

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>0.043</td>
<td>2</td>
<td>0.022</td>
<td>1.228</td>
<td>0.296</td>
</tr>
<tr>
<td>Within Groups</td>
<td>2.576</td>
<td>147</td>
<td>0.018</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2.619</td>
<td>149</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>2.029</td>
<td>2</td>
<td>1.014</td>
<td>39.027</td>
<td>0.000**</td>
</tr>
<tr>
<td>Within Groups</td>
<td>3.821</td>
<td>147</td>
<td>0.026</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.849</td>
<td>149</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*High significant difference at $p \leq 0.01$
Duncan's Multiple Analysis Range Test in Table (3) was done to determine which group had the highest value of surface roughness results. Groups were non-homogenously distributed as the silver diamine group had the minimum mean value followed by fluoride varnish and the maximum mean value of surface roughness belonged to the control group of deionized water.

**Table (3):** Duncan's Multiple Analysis Range Test for groups after pH cycle.

<table>
<thead>
<tr>
<th>groups</th>
<th>N</th>
<th>Subset for alpha = 0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDF2</td>
<td>50</td>
<td>0.6944</td>
</tr>
<tr>
<td>V2</td>
<td>50</td>
<td>0.7668</td>
</tr>
<tr>
<td>D2</td>
<td>50</td>
<td>0.9692</td>
</tr>
<tr>
<td>Sig.</td>
<td>1</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Significant difference at \( p \leq 0.01 \)

**DISCUSSION**

Dental caries is strongly associated with the dynamic process of demineralization and remineralization in the enamel surface. Moreover, the loss and addition of minerals that occurred during the process of demineralization and remineralization affect the roughness of enamel. After demineralization, enamel roughness increased. On the contrary, remineralization causes decreased enamel roughness. Therefore, enamel surface roughness is considered as one of the determinants describing the effects of remineralization and demineralization process on enamel surfaces \(^{(28)}\).

The use of profilometry in the current study has an advantage of exactly measuring the surface roughness, without the need for additional quantitative analysis. The surface roughness of the enamel could be analyzed by the profilometric method, which was considered as an effective quantitative evaluation \(^{(29)}\).

The null hypothesis of the current study was rejected because there were differences between surface roughness of sound and demineralized enamel of samples of primary teeth after application of different remineralizing agents. In the present study, we used demineralizing solution, which was prepared similar to preceding protocol \(^{(22,23)}\). In the current study the proposing idea that acid diffuses into the tooth structure and dissolves the carbonated hydroxyapatite, resulting in a demineralized porous enamel resembling**
carious like lesions and this will be accompanied by increase surface roughness of the tooth. Deciduous enamel is also inclined to the progression of dental caries, which occurs about 1.5 times faster than in enamel of permanent teeth. In contrast, it is important to accentuate that despite these disadvantages about permanent teeth, deciduous teeth are more sensitive to fluoride treatment. This may be explained by the greater permeability of the deciduous teeth enamel, which is about 150 times greater than in permanent teeth enamel, permitting the fluoride diffusion.

When comparing with previous studies the using of artificial saliva have shown that artificial saliva does not affect on the surface roughness of enamel.

ANOVA test at the beginning of the study is necessary to be sure there was no significant difference among groups before addition of any test materials and the starting of PH cycle demanded for production of enamel caries.

According to current study there was a minimum increase in the surface roughness of silver diamine fluoride group followed by fluoride varnish group and the maximum increase in surface roughness belonged to the control group of deionized water.

Results also showed no significant difference among groups before PH cycle while after the addition of test materials and introduction into PH cycle, there was highly significant difference among groups. On the other hand, Duncan Multiple Analysis Rang Test was done to determine which group had the highest value of surface roughness, showed that groups had non-homogenously distributed surface roughness as the silver diamine group had the minimum mean value followed by fluoride varnish and the maximum mean value of surface roughness belonged to the control group of deionized water.

According to the obtained results, we observe that the silver diamine fluoride group was better than fluoride varnish in preserving the surface characteristics of enamel of primary dentition and both remineralizing agents were better than the control group of deionized water. This could agree with the conclusion of study done by Ainoosh et al., 2020 who concluded that SDF was effective in reducing dental erosion on both enamel and dentin substrates, while for dental erosion–abrasion, SDF was effective only on dentine. The exact role of silver diamine fluoride in crystal formation is still unknown, however, the reduction of acid solubility of enamel thought to be due to the formation of fluorohydroxyapatite crystals during the reaction of SDF with calcium and phosphate, in addition to the formation of silver chloride. Other researchers declared that the inhibitory mechanism thought to be due to the F- released from the topically treated enamel and the formation of a protective barrier composed of
silver phosphate (Ag3Po4), calcium fluoride (CaF2) and fluorohydroxy apatite crystals (FHA). Additionally, the inhibitory efficacy of topical treatment with silver fluoride increased with increasing concentration (35).

CONCLUSIONS
Silver diamine fluoride solution was significantly better than fluoride varnish in preserving surface characteristics of enamel of primary dentition as reduce of surface roughness. On the other hand, silver fluoride showed the best effect against caries of the primary teeth compared to other conventional fluoride treatments.

REFERENCES


The Effect of Fluoride on Roughness of Primary Teeth Enamel


