An In-vitro Comparative Study of Fluoride Varnish and Two Calcium-Containing Fluoride Products on the Remineralization of Primary Teeth Enamel

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Abstract

Objectives: To compare the effectiveness of fluoride varnish and two calcium-based fluoride products on the remineralization of primary teeth enamel.

Materials and Methods: Surface-microhardness (SMH) of 36 extracted anterior primary teeth was measured by Vickers test (50gf/5 seconds) to provide a baseline for later comparisons. All teeth were immersed in demineralizing solution for 96 hours to create caries-like lesions and SMH was determined for the artificially-induced caries. The teeth were randomly assigned to three groups consisting of 5% fluoride varnish once daily/10 seconds, Clinpro™ 5000 toothpaste once daily/2 minutes, and Remin Pro cream once daily/3 minutes for 28 days. All specimens were kept in artificial saliva with pH cycling during the study period. After remineralization, SMH was evaluated for the last time. Data were analyzed by one-way ANOVA, Mauchly's sphericity, and RM-ANOVA with Bonferroni correction for inter-and-intra-group comparisons at the three stages of the study.

Results: Neither the baseline SMH nor the SMH of the artificially created caries showed significant differences among the samples (P>0.05). The post-treatment SMH was highest in the Clinpro group (296.4±73.1kgf/mm²), followed by Remin Pro (283.8±119.3kgf/mm²), and varnish (270.9±78.3 kgf/mm²). There was no significant difference among the groups after treatment (P>0.05). We also did not observe a significant difference among the three different study stages (P>0.05).

Conclusion: Within the limitations of this in-vitro study, daily application of low fluoride-calcium compound seems to be as effective as the professional use of fluoride varnish or high-content fluoride toothpaste in remineralizing initial caries of primary teeth.

Keywords: Dental Caries; Tooth Remineralization; Tooth, Deciduous

Introduction

The new direction of modern dentistry is to preserve or remineralize tooth structure especially in younger patients. Remineralization of incipient caries is a part of life-long caries management in dentistry, and proposes a fast, painless approach to substitute operative and surgical procedures in pediatric patients [1,2]. Remineralization is the process of substitution of lost minerals (calcium, phosphate) into the demineralized tooth tissue. It aims to decelerate or reverse the progress of early, non-cavitated caries in enamel or in some instances in the outer one third of dentin [3].
Gross body of evidence supports the efficacy of fluoride varnish in remineralization of early carious lesions in a number of ways, one of which is to decrease the threshold of enamel dissolution from pH 5.5-6 to 4.5 by replacing the demineralized hydroxyapatite with fluoro-hydroxyapatite [3-5]. Fluoride varnish is known as the best current substance for comparison with other fluoride and non-fluoride products. It has been used for both prevention and management of initial caries. However, the amount of fluoride needed for remineralization is higher than that required for prevention and it may increase the risk of fluorosis or have toxic effects in young children [6,7].

A more recent trend in dentistry is to add calcium and phosphorus ions to fluoride products to enhance their remineralizing ability. Several combinatory products of fluoride and other remineralizing agents have been introduced in recent years that claim to be an effective substance for preventing tooth caries as well as treating initial caries in children. The probable mechanism of action has been attributed to the ability to provide more fluoride and increase remineralization. This increase has been claimed to occur not only in the superficial layer but also in the inner parts of the enamel due to the presence of functionalized β-tricalcium phosphate (fTCP) and hydroxyapatite, or as a result of calcium ion transport enhancement [1,8-10].

To determine the effects of a mineralizing agent on enamel, the hardness or microhardness of the tooth surface is measured. This index indicates the resistance of the material to deformation, scratches and abrasion on its surface. Hardness is classified into two main types: scratch resistance and resistance to indenters. In dentistry, the type of submersible resistance has been used to measure microhardness, the most common of which is Vickers measurement.

The progression of caries in primary teeth is faster than permanent teeth, because of the thin and less-mineralized enamel layer, eating habits, and dependance on parents for oral hygiene procedures [1]. Many high-fluoride products have been introduced to manage incipient caries in adults. Due to the risk of fluorosis and toxic effects of fluoride, appropriate products to prevent and reverse caries in pediatric patients needs research. Limited studies have assessed the effect of novel remineralizing calcium-containing fluoride compounds on primary teeth [1,10]. The present study aimed to compare two calcium containing-fluoride products and fluoride varnish on the remineralization of primary teeth enamel. Our null hypothesis was that the amounts of microhardness of deciduous teeth do not vary following application of sodium fluoride varnish, Clinpro 5000, or Remin Pro.

**MATERIALS AND METHODS**

**Preparation of samples and measurement of initial microhardness**

The ethical approval of this study was obtained from the Ethical Board of Azad Dental School (IR.IAU.DENTAL.REC.1399.058). Sample size was calculated according to the results of Haghgoo et al., [11] using one-way ANOVA in PASS 11 software. Thirty-six anterior deciduous teeth were divided into three groups (N=12, each) by random assignment. First, freshly extracted teeth were examined under a stereo-microscope, (Nikon SMZ1000, Nikon Corp., Tokyo, Japan) to select teeth without cracks or hypoplastic enamel. The teeth were cleaned from debris, decontaminated, and kept in 2% formaldehyde solution for 30 days at room temperature [12-14]. They were then polished with pumice powder and distilled water, and embedded in self-polymerizing acrylic resin (Acopars100, Tehran, Iran) with their labial surfaces upward. All samples were abraded with silicon carbide abrasive paper number 600 followed by numbers 800 and 2400, so that the labial surface of the tooth was level with the acrylic surface [15].

In the next step, the initial SMH values of the teeth were measured using Vickers test (Bareiss Prügerätebau GmbH, D-89610. Oberdischingen, Germany) by applying vertical loads of 50g for 5 seconds on three points on the surface [15].

The average of the three readings was taken as the mean Vickers hardness number (kgf/mm²). The indentations were spaced 100um from each other to minimize the risk of interactions and crack propagation [15,11].
Creating demineralized enamel and determining microhardness of the artificially induced caries

To create demineralized enamel, the samples were submerged in 50ml demineralizing solution containing 0.1mM lactic acid, 3mM CaCl₂, 3mM KH₂PO₄ and 0.2% guar gum (pH 4.5). The final pH was adjusted to 4.5 using 50% sodium hydroxide. This solution was changed every 48 hours with fresh solution. After 96 hours in the demineralizing solution, the samples were washed with deionized water for 20 seconds, and dried followed by measurement of microhardness values [15, 7]. After demineralized enamel lesions were created, the specimens were subjected to pH cycling over a 28-day period to simulate the oral environment, during which three remineralizing compounds (Table 1) were randomly applied to the teeth by a micro brush.

pH cycling procedure
First, the samples were placed in a demineralizing solution containing 1.5mM CaCl₂, 0.9Mm KH₂PO₄, and 50mM acetic acid (pH=5), 6 times a day for 30 minutes each time. After each demineralization cycle, the samples were immersed in remineralizing solution for 2.5 hours, containing 1.5MM CaCl₂, 0.9MM KH₂PO₄, 130Mm KCl and 20Mm HEPES (pH=7). After the remineralization process, all samples were immersed in fresh artificial saliva overnight [8]. The method workflow is illustrated in Figure 1.

Table 1. List of remineralizing agents used in the experimental groups

<table>
<thead>
<tr>
<th>Name</th>
<th>Manufacturer</th>
<th>Fluoride compound and concentration</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoride varnish</td>
<td>Centrix USA</td>
<td>Sodium fluoride 5% (22,600 PPM F-)</td>
<td>Sodium fluoride, ethyl alcohol, methyl salicylate and hydrogenated rosin</td>
</tr>
<tr>
<td>Remin Pro topical cream</td>
<td>VOCO Germany</td>
<td>Sodium fluoride (1450 PPM F-)</td>
<td>Hydroxyapatite and xylitol</td>
</tr>
<tr>
<td>Clinpro™ 5000 toothpaste</td>
<td>3M ESPE</td>
<td>Sodium F 1/1% (5000 PPM F-)</td>
<td>Tricalcium phosphate (less than 2%), 40% -30% water, 30% -20% crystallized sorbitol, 10% -1% glycerin, 10% -5% amorphous silica, 20% -10% Synthetic amorphous silica, 5% -1% Polyethylene-polypropylene glycol, 5% -1% Polyethylene glycol, 20% -1% NaF, Titanium dioxide, Saccharide, Saccharide Sodium and sodium lauryl sulfate</td>
</tr>
</tbody>
</table>
After applying the treatment protocols, SMH values were measured in conditions identical to the SMH evaluations in our previous steps. **Statistical Analysis**

Comparisons were performed between SMH obtained at baseline, SMH of the induced caries, and post-treatment SMH values in each group and between the three groups in each stage.

**RESULTS**

Normal distribution of microhardness was confirmed by the Shapiro-Wilk test (P>0.05). As presented in Table 2 no statistically significant difference was seen between the experimental groups in any of the three experimental stages. Within-group comparison was performed using repeated measure ANOVA. For this purpose, first, the Mauchly’s test of sphericity was used. Due to sphericity violation (P<0.001, \( \varepsilon = 0.50 \)), the lower-bound correction in ANOVA was selected. The results showed a significant difference between SMH measured at baseline, SMH of the created caries, and post-treatment SMH in all three study groups (P<0.05). Binary comparisons (Bonferroni correction) showed that the baseline and post-treatment SMH values were similar (P>0.05), indicating the efficacy of all three compounds. SMH significantly decreased after demineralization, which simulates the caries process (P<0.05). According to Kruskal-Wallis test, the mean±standard deviation of changes between final and baseline SMH values in the Clinpro (99.5±46.4 kgf/mm²), Remin Pro (84.5±38.2 kgf/mm²), and varnish (86.8±26.3 kgf/mm²) were not significantly different from each other (P=0.88, H=0.23)

**Table 2. Comparisons of surface microhardness (kgf/mm²) within and between experimental groups**

<table>
<thead>
<tr>
<th></th>
<th>Mean ± standard deviation</th>
<th>Baseline</th>
<th>Artificially-induced caries</th>
<th>Post-treatment</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinpro 5000</strong></td>
<td></td>
<td>324.9±77.4</td>
<td>128.8±44.9</td>
<td>296.4±73.1</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Remin Pro</strong></td>
<td></td>
<td>340.7±28.2</td>
<td>98.9±51.7</td>
<td>283.8±119.3</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Fluoride Varnish</strong></td>
<td></td>
<td>330.9±98.2</td>
<td>86.5±30.1</td>
<td>270.9±78.3</td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td></td>
<td>0.87</td>
<td>0.61</td>
<td>0.79</td>
<td></td>
</tr>
</tbody>
</table>

*Within-groups: lower bound method in repeated measure ANOVA
**Between-groups: ANOVA
Also, due to the non-identical initial values of microhardness, despite the lack of significant differences between the groups, estimated marginal mean was calculated by ANOVA after adjusting the primary SMH. The results indicated the similar reciprocal effect of baseline SMH and three experimental materials (P=0.55, F=0.59).

**DISCUSSION**

This in-vitro study compared the remineralizing effect of Clinpro 5000 toothpaste, Remin Pro cream (1450 PPM fluoride ion), and 5% sodium fluoride varnish (22600 ppm) on the surface microhardness of demineralized deciduous tooth enamel. Based on the results, no significant difference was observed between the baseline and post-treatment SMH after application of either of the products. This finding was consistent with other in-vitro studies in primary and permanent teeth [10, 20-23]. Sahiti et al., [22] in a similar study in permanent teeth, compared the SMH of Remin Pro and Clinpro and did not find a significant difference in remineralization between the materials, although Remin Pro resulted in higher SMH values. Nourolahian et al., [10] reported equivalent remineralization effects of Remin Pro and APF 1.23% gel in primary molars. Cherian et al., [21] evaluated the remineralizing potential of Remin Pro, and Clinpro using scanning electron microscope and energy dispersive X-ray on human premolars. Their results indicated a superior efficacy of Remin Pro in comparison to Clinpro.

The findings obtained in the present study may be explained by the mechanism of action of the studied materials [24]. In contrast to our results, Valian et al., [25] reported superior remineralization of neutral sodium fluoride gel compared to Remin Pro. They evaluated the remineralization process after using acidic beverages, which may explain the difference between our findings.

Fluoride products have the highest level of supporting evidence for remineralization of incipient lesions. One of the shortcomings of both high- and low-dose topical fluorides is related to limited subsurface remineralization due to blocking surface pores and inhibiting ion exchange to subsurface layers [6]. The phenomena of surface-only remineralization have been called fluoride syndrome or occult caries, which also inhibit dentin remineralization [26]. Moreover, the choking-off effect of high-fluoride topical products decreases absorption of fluoride, calcium, and phosphate ions by demineralized enamel [6]. The novel remineralization technology is comprised of two main categories: biomimetic regenerative systems such as nanohydroxyapatite compounds, and fluoride booster systems e.g., functionalized β-tricalcium phosphate [2]. Both of these systems have been used in our study materials. The mechanism of action of Remin Pro is dependent upon its constituent materials which are 1450ppm fluoride, nanohydroxyapatite, and xylitol. Synthetic nanohydroxyapatite is a bioactive and bio-compatible material that has similar crystallite morphology and structure to enamel. The remineralizing function is proposed to be a result of its capability of filling porosities in the enamel, producing a new enamel layer, or preserving minerals around the enamel surface and subsurface [2,26,27]. An equivalent or superior remineralization potential of nanohydroxyapatite toothpastes in comparison to fluoride toothpastes has been reported in previous in-vitro studies [6]. Nanohydroxyapatite-containing toothpastes facilitate remineralization by elevating the Ca²⁺ and PO₄³⁻ levels in saliva, biofilm, and tooth surface. [6,28]. The other study material was Clinpro 5000 toothpaste, the main component of which is functionalized β-tricalcium phosphate (f-TCP). In its functionalized state, f-TCP acts as a vehicle for targeted transport of ions to tooth surface, while simultaneously boosts the fluoride effect [2]. Similar effectiveness of Clinpro 5000 with Remin Pro, and NaF varnish in our study was in agreement with clinical studies of the three products [29].

The presence of fluoride ions in saliva promotes the incorporation of calcium and phosphate ions into the crystal lattice. Therefore, fluoride products containing calcium, phosphate, or hydroxyapatite could
increase the efficacy of either compound while lowering the fluoride concentration [2,6]. The current fluoride toothpaste recommendation for preschool children, is supervised tooth-brushing with a minimum of 1000ppm F-. The relatively similar in-vitro remineralizing effect of Remin Pro cream, in comparison to high fluoride products such as fluoride varnish and Clinpro 5000, and the safety of varying doses of hydroxyapatite in clinical studies, might encourage researchers to evaluate its clinical use to remineralizing enamel lesions of primary dentition [3,29].

Due to the importance of the surface layer in the progression of caries, the evaluation of changes in specific areas is very important. Hence, the SMH test was used as the method of choice for evaluating the remineralizing effect of the studied materials. The advantage of using hardness testing is its high accuracy and its submersible type [15,23]. One of the strengths of present study was to calculate the impact of confounding variables by adjusting initial microhardness (despite non-significant statistical difference) by estimating marginal means to confirm the similar impact of three remineralizing agents on SMH. Artificial carious lesions such as what we used in the present study have all the histological characteristics of natural dental caries and have been successfully used in enamel remineralization researches in-vitro [7,10,29]. These lesions are more homogeneous than natural dental caries and are therefore a valid laboratory model for determining the degree of demineralization/remineralization [30]. There are different models for simulating the caries process. In the present study, a laboratory pH-cycling model was used to simulate the natural process of caries lesions in the oral environment [24]. This cycle of demineralization and remineralization mimics changes in the oral environment during food intake [9].

The current investigation was carried out in-vitro which has limitations compared to oral conditions. There are host-related variables such as tooth and pellicle mineral concentrations or plaque-forming conditions such as microorganisms, which can affect the amount of demineralization. Based on previous research there is insufficient evidence to support the advantage of antiplaque properties of high fluoride toothpastes on remineralization [29,31]. Comprehensive clinical research will be needed for recommendation of such products.

CONCLUSION

Within the limitations of this laboratory study, it could be concluded that the observed remineralization effect of fluoride varnish, Clinpro 5000 toothpaste, and Remin Pro cream was similar. A product with similar effectiveness but less fluoride content is a better option in terms of safety.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES


