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Remineralization and Aesthetic Effect of Three Agents on Artificial White Spot Lesions: An in Vitro Study

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Abstract

Objective: To evaluate the remineralization potential of three different dentifrices.

Materials and methods: Premolars (n = 80) were randomly allocated into four groups (n = 20), group A, casein phosphopeptide–amorphous calcium phosphate; group B, nano silver fluoride; group C, sodium fluoride; and group D, artificial saliva. Specimens were subjected to a pH cycling regime for 21 days. DIAGNOdent recorded the mineral profile, and spectrophotometer evaluated the aesthetic effect. The data were analyzed using (analysis of variance) and Turkey’s post-hoc multiple comparison tests at a significance level of P value less than 0.05.

Results: Regarding the baseline stage, improvement of the median of DIAGNOdent readings was not significant, six [3–11] (P = 1.0). In postlesion, the median of group A was 14 [11–18], in comparison to nano sodium fluoride the median value of DIAGNOdent readings was 15 [11–19], compared to sodium fluoride group 14 [12–17], and in the control group it was the same 14 [12–17] (P = 0.4). After remineralization the mean value of group A was six [4–7], while in group B it was three [1–5], compared to the group C it was eight [6–11], and in the control group it was 15 [11–19] (P < 0.001). It was found that there was a statistically significant difference in the mean values of spectrophotometer readings (P = 0.001). However, there was no significant difference after remineralization compared to baseline, except in the control group (P2<0.05).

Conclusions: Within this in-vitro study; group B, followed by A, showed improvements in aesthetic appearance. However, group C demonstrated less remineralization potential in comparison to other groups.

Keywords: Nano silver fluoride, Remineralization, White spot lesions

Introduction

The prevalence of white spot lesions (WSLs) ranges from 25 to 46 %. WSLs can be detected as white opaque lesions after air-drying the teeth. On the other hand, remineralization, which is one of the minimal invasive dentistry approaches takes place through the mineral ions present in the saliva and occurs only in the superficial layer of WSLs.1

Fluoride is the most commonly used substance for remineralization. The scientific basis for the use of fluoride in fighting against caries is that fluoride ions can penetrate the crystalline structure of dental hard tissues, decrease their solubility and confer acid resistance. Additionally, nonfluoride agents have a protective effect against the development of dental caries, and has been a novel concept in remineralization.2

Casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) gives a new area to preventive dentistry. Providing a pool of calcium and phosphate which can maintain the supersaturation of saliva. Since CPP–ACP can stabilize calcium and phosphate in the solution, it can also help in the buffering of plaque pH and so calcium and phosphate level in plaque is increased.3

Nano silver fluoride (NSF)-based preparations are gaining popularity as excellent antibacterial, antifungal, and antiviral agents. The action of NSF in the arrest of caries can be demonstrated by the
synergism of the components, chitosan, and fluoride formulation. Silver is typically used nitrates form to cause antimicrobial effects. Therefore, the goal of this study was to evaluate and compare the effectiveness of three remineralizing agents: CPP–ACP, NSF, and sodium fluoride (NaF), in comparison to the control group: artificial saliva on enamel of WSLs. The null hypothesis was that there is no difference in remineralizing effect of these materials.

Materials and methods

This study was conducted after the approval of the Ethical Committee of Dental research, at the Faculty of Dentistry, Mansoura University, under the coding number of A08120520.

The premolars were extracted for orthodontic purpose, followed by immersion in Thymol solution (C₁₀H₁₄O, at 0.9 g/l from 1 to 5 %; in formalin instead) prepared by Pharmaceutics Department, Faculty of Pharmacy, Mansoura University. They had been collected from the Clinics of Oral Surgery Department, Faculty of Dentistry, Mansoura University, after obtaining the approval of the patient to use their teeth for the research purpose.

Eighty samples of human permanent premolars (20 samples in each group) were used in this study. Group I: CPP–ACP (GC tooth mousse, topical crème with bio available calcium and phosphate; Recaldent Milk Protein, GC America Inc; Alsip, IL, USA), group II: NSF (prepared by Nano Gat, Quality Control Department, MaterialDatasheet, Nasr City, Cairo, Egypt), group III: NaF (1450 ppm fluoride, zinc sulfate; Colgate, Germany), and group IV: control group (artificial saliva, is composed of methyl-p-hydroxybenzoate, sodium carboxymethyl cellulose, and calcium chloride). The pH was adjusted to 6.75 by the Pharmaceutics Department, Faculty of Pharmacy, Mansoura University.

Fig. 1. pH cycling.
Tests applied in this study were mineral profile assessment by DIAGNOdent and aesthetic analysis using Spectrophotometer. The assessment of the mineral content of all samples was done at three stages baseline stage, postlesion stage, and after remineralization of all four test groups by DIAGNOdent pen (Kavo, Germany) device. Additionally, the aesthetic change was evaluated, using the spectrophotometer (shademodel v 505 h, Germany) fixed Prosthodontics Department, Mansoura University.

Results

Data were tabulated, coded then analyzed using the computer program SPSS (IBM SPSS Statistics for Windows, v25; IBM Corp, Chicago IL, USA). In the statistical comparison between the different groups, the significance of difference was tested using:

Kruskal–Wallis test to compare between more than two different groups of numerical (nonparametric) data, followed by post-hoc Dunn’s test. Additionally, Friedman test used to compare between more than two related groups of numerical (nonparametric) data followed by post-hoc Dunn’s test. A P value less than 0.05 was considered statistically significant.

According to the DIAGNOdent hygienic pen values for the CPP–ACP group, the median value at baseline was 6 (3–11) which lies between the normal readings of DIAGNOdent, after demineralization the median was 14 (11–18) indicating the formation of initial WSLs. After remineralization, the median was 6 (4–7) which indicates the return to the normal range as shown in Table 1.

There was a statistically significant difference between CPP–ACP and other study groups after remineralization (P < 0.05). In the nano silver group, the median value at baseline was 6 (3–11) in the normal range, then after demineralization, it was 15 (11–19), and after remineralization, it was 3 (1–5) within the normal range. A statistically significant difference was obtained compared to other groups (Table 1).

Regarding the NaF group, the median value at baseline was 6 (3–11), then after lesion creation was 14 (12–17), and after remineralization was 8 (6–11). A statistically significant difference was found compared to other groups (P < 0.05) as shown in Table 1.

Regarding the control group, the median at baseline was 6 (3–11), after demineralization was 14 (12–17), and after remineralization was 15 (11–19) (P < 0.05) as shown in Table 1.

The remineralizing efficiency was significantly higher in the NSF group, followed by the CPP–ACP group, then the NaF group, and finally the control group.

Regarding the baseline stage, the median of all groups was the same 6 (3–11) (P = 1.0). While in the postlesion stage, the median value of the CPP–ACP group was 14 (11–18), in comparison to the NaF group the median value was 15 (11–19), compared to the NSF group 14 (12–17), and in the control group it was the same 14 (12–17) (P = 0.4). After remineralization treatment, the mean value of the CPP–ACP group was 6 (4–7), while in the NSF group, it was 3 (1–5), compared to the NaF group it was 8 (6–11), and in the control group it was 15 (11–19) (P < 0.001) as shown in Table 1.

The aesthetic effect in all groups showed a statistically significant difference in the mean values of spectrophotometer readings after remineralization, compared to the control group (P = 0.001). However, there was no significant effectiveness between the three test groups (P > 0.05), as shown in Table 2.

Discussion

In the current study, all premolars were kept in thymol solution to avoid any infection or storage

Table 1. Comparison of DIAGNOdent reading between all groups through the study stages.

<table>
<thead>
<tr>
<th></th>
<th>CPP–ACP (group I)</th>
<th>NSF (group II)</th>
<th>NaF (group III)</th>
<th>Control (group IV)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>6.0 (3.0–11.0)</td>
<td>6.0 (3.0–11.0)</td>
<td>6.0 (3.0–11.0)</td>
<td>6.0 (3.0–11.0)</td>
<td>1.0</td>
</tr>
<tr>
<td>Postlesion</td>
<td>14.0 (11.0–18.0)</td>
<td>15.0 (11.0–19.0)</td>
<td>14.0 (12.0–17.0)</td>
<td>14.0 (12.0–17.0)</td>
<td>0.4</td>
</tr>
<tr>
<td>After remineralization</td>
<td>6.0 (4.0–7.0)</td>
<td>3.0 (1.0–5.0)</td>
<td>8.0 (6.0–11.0)</td>
<td>15.0 (11.0–19.0)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>post-hoc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The test used Kruskal–Wallis followed by post-hoc Dunn’s.

P1: significance of control group versus CPP–ACP group.

P2: significance of control group versus NSF group.

P3: significance of control group versus NaF group.

Control, artificial saliva; CPP–ACP, casein phosphor peptide–amorphous calcium phosphate; NaF, sodium fluoride; NSF, nano silver fluoride.

*: P < 0.05.
Table 2. Comparison of the aesthetic effect between all groups through the study stages.

<table>
<thead>
<tr>
<th>Variables</th>
<th>CPP–ACP group I</th>
<th>NSF group II</th>
<th>NaF group III</th>
<th>Control group IV</th>
<th>ANOVA test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>3.00 ± 0.59</td>
<td>2.96 ± 0.74</td>
<td>2.99 ± 0.74</td>
<td>2.79 ± 0.69</td>
<td>0.19</td>
<td>0.89</td>
</tr>
<tr>
<td>Postlesion</td>
<td>15.01 ± 1.99</td>
<td>16.15 ± 2.48</td>
<td>15.41 ± 2.27</td>
<td>15.83 ± 2.30</td>
<td>0.47</td>
<td>0.69</td>
</tr>
<tr>
<td>Postremineralization</td>
<td>2.87 ± 0.51</td>
<td>2.61 ± 0.65</td>
<td>2.87 ± 0.71</td>
<td>8.56 ± 2.14</td>
<td>57.83</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Post-hoc</td>
<td>P1 = 0.69</td>
<td>P1 = 0.99</td>
<td>P2 = 0.96</td>
<td>P1 = 0.001*</td>
<td>P2 = 0.001*</td>
<td>P3 = 0.001*</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SD.  
Control, artificial saliva; CPP–ACP, casein phosphopeptide–amorphous calcium phosphate; NaF, sodium fluoride; NSF, nano silver fluoride.  
Test used: one way analysis of variance followed by post-hoc Tukey test.  
P1: significance versus CPP–ACP group.  
P2: significance versus NSF group.  
P3: significance versus NaF group.  
* Significance P value less than 0.05.

mistake, till the study had ended. This was in line with Dhanya et al. who reported that thymol is widely used to store teeth before research performance, for clinical specimen fixation. This study used a pH cycling model, which conformed with the pH-cycling protocol of Buzalaf et al. In the current study, the pH-cycling process was carried out for 21 days in line with Singhal and Rai as in the adopted protocol.

This pH cycling model has been successfully utilized to show the remineralizing effect of these remineralizing materials since it mimics in-vivo high-risk category situations that pH-cycling protocol entails exposure of dental substrates to a series of demineralization and remineralization. This was in line with Nakamura et al.

Hence it is necessary to prevent the progression of dental caries at an early stage, rather than to develop treatment strategies for progressive dental caries. Enamel is subjected to innumerable cycles of demineralization and remineralization throughout its lifetime, which controls the progression or reversal of caries. In contrast with another study which was conducted for seven days only, and accomplished by Premnath et al.

In the present study, DIAGNODent was used to evaluate the remineralizing efficacy as finding a modality that would not be dependent on patient collaboration leading to a more accurate evaluation of the caries situation. DIAGNODent is a simple device to be used in a short time (a few seconds) for caries detection and quantification. It has been introduced recently in quantifying smooth surface caries in line with Aldhaian et al.

The results of the present study revealed a statistically significant difference between all groups in the median change of the DIAGNODent measurements (P < 0.05). CPP has a significant ability to stabilize calcium and phosphorus ions and acts as a delivery carrier to significantly increase calcium and phosphorus levels in plaque. CPP–ACP buffers free calcium and phosphate ion activity, thereby helping to maintain the supersaturated state of tooth enamel, reduce demineralization and promote remineralization. Free calcium and phosphate ions are removed from CPP, enter the enamel, and are reformed onto apatite crystals. CPP–ACP can be used to treat WSLs, and low mineralized enamel. Silver nanoparticles can penetrate carious lesions and attach to hydroxyapatite crystals, small silver nanoparticles are prone to releasing silver ions due to their larger ratios of surface to volume. In addition, silver nanomaterials can preserve exposed collagen in carious teeth in the oral environment. Then, the preserved collagen can act as a scaffold for the deposition of a mineral crystal and the prevention of calcium and phosphate’s further diffusion. NaF can unite with calcium ions and hydrogen phosphate ions to form fluorapatite and fluorhydroxyapatite. Therefore, researchers proposed using CPP–ACP, NSF, and NaF to promote the remineralization of enamel. This finding was in line with Brunton et al., Jablonski-Momeni and Heinzel-Gutenbrunner, and Oliveira et al.

From the result of our study, we found that the NSF group was the highest, significant remineralization agent when compared to the control group. The protective effect of NSF could be explained by the very small particle size of silver nano particles which facilitated the penetration of the material into the enamel structure maximizing its effect. This might be attributed to the formation of a highly mineralized surface layer that resulted from direct contact with
materials having high fluoride content. Silver ions could infiltrate into carious lesions and precipitate there. This could result in enamel hardening. The NSF group showed uniform acicular crystals which covered the enamel pores, as shown in aesthetic analysis, using spectrophotometer test. Its organized microstructure seemed like a woven unit protecting the enamel from demineralization attack. This was in line with Nozari et al. In contrast with Akıylidiz et al. Therefore, it has the potential to replace NaF therapy substance, additionally, NaF showed the least values of remineralization. Although NaF has shown a lower percentage of mineralization variation, no definite protective layer was observed in the control group except for minor crystals caused by the remineralization solution, as shown in aesthetic analysis, using spectrophotometer test. This result could be related to the prolonged remineralization caused by globular particles (larger size than nanosize scale), that the remineralization process occurs much more slowly and in two phases. This was in agreement with Ionta et al. Regarding the aesthetic assessment, it revealed that NSF, followed by CPP–ACP has a better aesthetic effect compared to NaF. It was found that there was a statistically significant difference in the mean values of spectrophotometer readings after remineralization in all study groups. The aesthetic effect of NSF could be explained by the very small particle size of silver nano particles which facilitated the penetration of the material into the enamel structure maximizing its aesthetic effect, to decrease the extent of the body of the lesion in comparison to the control group. Revealed that the enamel treated with NSF showed shallower lesion depth and the caries were arrested, forming a highly mineralized layer on the surface of the caries-like lesion. Additionally, CPP–ACP has an excellent ability to repair and smooth the surface of enamel, ultimately acquiring desirable aesthetic effects. This result can be attributed to the mineralization induction of CPP–ACP: after the localization of ACP at the enamel surface, free calcium and phosphate ions were buffered, thereby helping to maintain a state of supersaturation concerning tooth minerals, depressing enamel demineralization, and promoting remineralization. This was in agreement with Kim and Kim.

Conclusions

Within this in-vitro study, NSF and CPP–ACP containing dentifrice were effective in remineralizing lesions of enamel. However, NaF demonstrated less remineralization potential in comparison to dentifrices containing NSF and CPP–ACP.

Conflicts of interest

There are no conflicts of interest.

References