Nano Silver Remineralization and Aesthetic Effect on Artificial White Spot Lesions: an in vitro study

Hadeer Wael  
Mansoura University

salwa hegazy (✉ ahmed1alaa@yahoo.com)  
Mansoura University

Rasha Ibrahim  
Mansoura University

Research Article

Keywords: NSF, Remineralization, WSLs, CPP-ACP

Posted Date: January 27th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2448034/v1

License: ☝️ ☢️ This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
Abstract

Background: Evaluating the remineralization potential on artificial enamel caries of three different dentifrices containing, Casein Phospho peptide- Amorphous Calcium Phosphate (CPP-ACP), Nano Silver Fluoride (NSF), and Sodium Fluoride (NaF), in comparison to Artificial Saliva (ASS).

Methods: A standardized demineralization protocol was used on sound extracted human premolars (n=80) in order to create artificial carious-like lesions. Premolars were sectioned longitudinally. According to the type of treatment group, the teeth were randomly allocated into four equally sized groups (n=20). Group A, was treated with Casein Phospho Peptide- Amorphous Calcium Phosphate (CPP-ACP); Group B, was treated with Nano Silver Fluoride (NSF); and Group C, was treated with Sodium Fluoride (NaF); group D, was treated with Artificial Saliva (ASS), as control group. The specimens were then subjected to standardized pH cycling regime for 21 days. The (Diagnodent laser) test was utilized to record the mineral profile in all groups; the (Spectrophotometer) test was used to evaluate the aesthetic effect of remineralizing agents, as well. The assessment was done in three stages; (Baseline stage), (Lesion-creation stage), and (after-remineralization stage) finally. The data were statistically analyzed using a two-way analysis of variance (ANOVA) and Turkey’s post-hoc multiple comparison tests at a significance level of p<0.05.

Results: Nano Silver Fluoride (NSF): Group B significantly increased the mineral profile and aesthetic appearance after artificial carious-like creation stage. As well as, Group B: Nano Silver Fluoride (NSF) reported the highest percentage change in mineral profile values and aesthetic appearance followed closely by Group A: Casein Phospho Peptide- Amorphous Calcium Phosphate (CPP-ACP), and Group C: Sodium Fluoride (NaF); respectively the most predominant failure was in control group.

Conclusion: Within this in-vitro study; Nano Silver Fluoride (NSF), and Casein Phospho Peptide- Amorphous Calcium Phosphate (CPP-ACP) containing dentifrice were effective in remineralizing lesions of enamel, as well as improving the aesthetic appearance. However, Sodium Fluoride (NaF) demonstrated less remineralization potential in comparison to dentifrices containing Nano Silver Fluoride (NSF), and Casein Phospho Peptide- Amorphous Calcium Phosphate (CPP-ACP).

Introduction

The prevalence of white spot lesions (WSLs) ranges from 25% to 46%. White spot lesions can be detected as white opaque lesions after air-drying the teeth. On the other hand, remineralization, which is a one of the Minimal Invasive Dentistry (MID) approaches takes place through the mineral ions present in the saliva, 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 into the crystalline structure of dental hard tissues, decrease their solubility and confer acid resistance. Additionally, Non-fluoride agents have
a protective effect against the development of dental caries, has been a novel concept in remineralization. ²

Casein phosphopeptide–amorphous calcium phosphate system like (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–based preparations are gaining popularity as excellent anti-bacterial, anti-fungal and anti-viral 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.⁴

Therefore, the goal of this study was to evaluate and compare the effectiveness of three remineralizing agents: CPP-ACP, NSF, and 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.

Aim of the study:

This study was conducted to:

1. Investigate the efficacy of CPP-ACP, and Nano Silver Fluoride in comparison to sodium fluoride on remineralization of artificially induced enamel white spot lesions, using DIAGNOdent mineral profile records.

2. Examine the Esthetic effect of remineralized enamel surface after using the previous tested materials using Spectrophotometer (Vita Easyshade).

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. 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 in the research purpose.

Eighty samples of human permanent premolars (20 samples in each group) were used in this study. Group I: CPP-ACP, group II: Nano Silver Fluoride, group III: Sodium Fluoride, group IV: Control group. Each of the samples was individually in the demineralizing solution for continuous 96hr to create artificial caries lesions. The treatment materials for group A, B, and C were applied using microbrush two times a day for 5 minutes each time, as shown in figure (1). Group D was considered passive control group, no
remineralizing agents was applied, it follow pH cycling like other groups except application of treatment materials.

The assessment of mineral content of all samples was done at three stages: baseline stage, post-lesion stage, and after remineralization of all four test groups by Diagnodent pen (Kavo Germany) device. Additionally, the esthetic change was evaluated using the spectrophotometer.

Results

Data were tabulated, coded then analyzed using the computer program SPSS. In the statistical comparison between the different groups, the significance of difference was tested using one of the following tests:

- **Kruskal wallis:** Used to compare between more than two different groups of numerical (non-parametric) data followed by post-hoc Dunn's.
- **Friedman:** Used to compare between more than two related groups of numerical (non-parametric) data followed by post-hoc Dunn's.

A $P$ value <0.05 was considered statistically significant.

1. **Mineral profile using Diagnodent**

   **Table (1) showing Comparison of diagnost dent reading between all groups.**

According to the DIAGNO 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, while after demineralization the median was $14 (11-18)$ indicating the formation of initial white spot lesions. After remineralization, the median was $6 (4-7)$ which indicate the return to the normal range. Regarding these results, there was a statistically significant difference between CPP-ACP and other study groups after remineralization ($p<0.05$). As showed in Table (1).

In the Nano silver group, the median value at baseline was $6 (3-11)$ in normal range, then after demineralization, it was $15 (11-19)$, and after remineralization, it was $3 (1-5)$ within normal range. A statistically significant difference was obtained compared to other groups as showed in Table (1).

In comparison with sodium fluoride the gold standard 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 showed 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 $15 (11-19)$. ($p<0.05$). Table (1).
The remineralizing efficiency was significantly higher in (NSF group), followed by (CPP-ACP group), then (sodium fluoride 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 post lesion stage the median value of CPP-ACP group was 14(11-18), in comparison to NSF group the median value was 15(11-19), compared to the NAF 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 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).

Table (1): Comparison of diagnotent reading between all groups.

<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>Post-lesion</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>P1=0.005*</td>
<td>P1=&lt;0.001*</td>
<td>P1=&lt;0.001*</td>
<td>P2=0.017*</td>
<td>P3=0.003*</td>
</tr>
</tbody>
</table>

Table (2) showed change in diagnotent reading in all groups.

The test used *Kruskal wallis followed by post-hoc Dunn’s*

**P1:** significance vs CPP-ACP Group

**P2:** significance vs NSF Group

**P3:** significance vs NAF Group

Table (2) showed change in diagnotent reading in all groups. It was found that there was a statistically significant difference between all groups in the change of the diagnotent measurements (difference between after remineralization reading and post lesion reading) (p< 0.05)
<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>Change (after-mineralization - Post-lesion)</td>
<td>-8.5(-13.0 - -4.0)</td>
<td>-13.0(-16.0 - -6.0)</td>
<td>-6.5(-9.0 - -3.0)</td>
<td>1.0(-5.0 - 5.0)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Post-hoc</td>
<td></td>
<td>P1=0.01*</td>
<td>P1= &lt;0.001*</td>
<td>P2=0.08</td>
<td>P1=&lt;0.001*</td>
</tr>
</tbody>
</table>

Data expressed as Median(Range)

P: Probability *: significance <0.05

Test used: Kruskal wallis followed by post-hoc Dunn’s

P1: significance vs CPP-ACP Group

P2: significance vs NSF Group

P3: significance vs NAF Group

Table (3): Comparison of the aesthetic effect between all groups

Table (3) showed comparison of the aesthetic effect in all groups. It was found that there was a statistically significant difference in mean values of Spectrophotometer reading after remineralization in all study groups compared to control group (P=0.001), as shown in fig (2).
<table>
<thead>
<tr>
<th>Variables</th>
<th>CPP-ACP Group</th>
<th>NSF Group</th>
<th>NAF Group</th>
<th>Control Group</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>Post-lesion</td>
<td>15.01±1.99</td>
<td></td>
<td></td>
<td>15.83±2.30</td>
<td>0.47</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16.15±2.48</td>
<td>15.41±2.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-remineralization</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>P1=0.001*</td>
<td>P2=0.96</td>
<td>P2=0.001*</td>
<td>P3=0.001*</td>
</tr>
</tbody>
</table>

Data expressed as Mean±SD

P:Probability    *:significance <0.05

Test used: One way ANOVA followed by post-hoc tukey

P1: significance vs CPP-ACPGroup

P2: significance vs NSFGroup

P3: significance vs NAF Group

Discussion

In current study all premolars were kept in thymol solution to avoid any infection or storage mistake, till the study had ended. This was in line with Dhananjaya KM, et al (2021). This study used a pH cycling model, which was in conformity with The pH – cycling protocol of Buzalf, et al. (2017). In the current study, the pH cycling process was carried out for 21 days. In line with Rajnish K, etal (2017), and Nakamura K, etal. (2021). In contrast with another study which was accomplished by Premnath Petal (2019) and conducted for seven days.
In the present study DIAGNOdent was used to evaluate the remineralizing efficacy as finding a modality which wouldn’t be dependent on patient collaboration lead to more accurate evaluation of the caries situation. DIAGNOdent is simple device to be used in a short time (few seconds) for caries detection and quantification has been introduced recently in quantifying smooth surface caries. In line with Braga et al (2018)\textsuperscript{10}

The results of the present study revealed that the CPPACP–group, and Nano-silver group were significantly different from the control. This finding was in line with Brunton PA et al (2013)\textsuperscript{11}, Wu et al. (2018)\textsuperscript{12}, and Oliveira et al., (2020)\textsuperscript{13}

From the result of our study, we found that Nano Silver Fluoride group was the highest significant remineralization agents when compared to the control group. This was in line with (Nozari et al., 2017)\textsuperscript{14}. In contrast with Akyildiz M et al. (2019)\textsuperscript{15}

The result of the current study showed that the positive control group (sodium Fluoride) showed the least values of remineralization. This was in agreement with Ionta FQ, et al (2014)\textsuperscript{16}

From our current result of spectrophotometer device for aesthetic assessment. The aesthetic effect revealed that NSF, CPP-ACP have better aesthetic effect compared to the sodium fluoride(control group). This was in accordance with Kim HE et al.,(2015)\textsuperscript{17}

Conclusion

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.

Recommendation

- More in –vitro studies are needed to evaluate the aesthetic effect of these novel remineralizing agents like NSF.
- More in-vivo studies are needed to support the remineralizing efficacy, and aesthetic effect of the novel remineralizing agents.

Declarations

Ethics approval and consent to participate

All procedure performed in this study, were carried out in accordance with relevant guidelines and regulations of Helsinki Declarations. All the experimental protocols were approved by the ethical committee of the Faculty of Dentistry, Mansoura University with reference number (A08120520). Teeth extracted for therapeutic reasons unrelated to the study were collected, with prior informed consent from
healthy individuals who were seeking dental care at the Outpatient Dental Clinic, Faculty of Dentistry at Mansoura University.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The data sets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

**Funding**

This research did not receive any specific funding.

**Authors contribution**

Conception any study design were done by HW. The tests were performed by HW. HW, SH and RI interpreted the statistical results. The manuscript was written by HW and revised by SH and RI. All authors read and approved the final manuscript.

**Acknowledgments**

Not applicable.

**References**


Figures
Figure 1

showing pH cycling regime
Figure 2

Box and Whiskers plot was used to show aesthetic analysis among different groups at different stages of the study by spectrophotometer.