

## Comparison of Self-Assembling Peptide with Different Remineralizing Agents on Demineralized Primary Molars: In-Vitro Study

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### Abstract:

**Objective:** Evaluating the remineralization potential of Self assembling peptide (P11-4), Nano-hydroxyapatite (Nano-HA) and Casein Phosphopeptide-Amorphous Calcium Phosphate-Fluoride (CPP-ACPF) using Scanning Electron Microscopy (SEM) and Energy dispersive X-ray Analysis (EDX). **Materials and Methods:** Forty enamel specimens of primary second molar were prepared and immersed in demineralizing solution for 96 hours to induce artificial carious lesions. Specimens were divided randomly according to remineralizing agents into four equal groups. The groups were named group A: control group (artificial saliva), group B: P11-4, group C: Nano-HA paste and group D: CPP-ACPF. Remineralization was carried out for a period of 21 days. Calcium and Phosphorous content was evaluated using EDX-SEM for all specimens. The data were collected and statistically analyzed using One Way ANOVA test. **Results:** The Calcium/Phosphorous ratio % after remineralization was significantly higher with Self assembling peptide and Nano-HA and the increase in CPP-ACPF was non-significant compared to control group. On SEM examination, there was a restoration of enamel surface with appearance of mineralized deposits. It was highly observed in group B and group C then group D and the lowest was in group A. **Conclusion:** Self-assembling peptide and Nano-hydroxyapatite were found to be more effective compared to CPP-ACPF in remineralization.

### Introduction:

Dental caries is a complex dynamic procedure, which begins from the first atomic-level of demineralization, develops to the white spot and frequently can lead to dentinal involvement resulting in cavitation. The equilibrium between demineralization and remineralization, as affected by protective factors and pathological factors, determines the fate of the lesion.<sup>1</sup>

Recently, scientists from the University of Leeds developed a patented technology for regeneration of enamel: The Curolox technology. Self-assembling peptide P11-4 is a rationally-designed peptide, the monomers of which undergo well characterized self-assembly into a biocompatible fibrillar scaffold in response to specific environmental triggers that mimics the enamel matrix. Around this matrix, enamel crystals are formed from calcium phosphate from the saliva.<sup>2-4</sup>

Nano-hydroxyapatite (Nano-HA) is considered one of the most biocompatible and bioactive materials.<sup>5</sup> Its nanoparticles have similarities to apatite crystals of natural enamel in morphology and crystal structure. Various in vitro studies have revealed superior remineralizing efficacy of Nano-hydroxyapatite over fluorides in permanent teeth.<sup>6-8</sup> However, few studies exist regarding the efficacy of these agents in primary teeth.

The use of milk and milk products, having a protective effect against the development of dental caries, has been a novel concept in remineralization. The anti-cariogenic properties of milk are due to the presence of casein, calcium and phosphate, which are responsible for resistance to acid dissolution.<sup>9,10</sup> Casein phosphopeptide amorphous calcium phosphate (CPP-ACP) is one of this

remineralizing agent that has been thoroughly researched and recognized. Fluoride incorporated into CPP-ACP is shown to have a higher remineralization potential.<sup>11</sup> It is worthy to evaluate and compare the remineralizing effect of these materials especially on primary teeth as there is a limited data regarding this issue.

### Materials and Methods:

This in vitro study protocol was approved by Ethical committee of the Faculty of Dentistry, Mansoura University with the code number: A02120520. Twenty primary second molar with sound buccal and lingual surfaces were collected. Teeth were extensively cleansed with an ultrasonic scaler and polished with pumice prophylaxis to remove debris.

Each tooth's radicular portion was removed, the coronal part of each tooth was sectioned mesiodistally into two halves using a low-speed diamond tipped disc. A total of 40 enamel specimens were produced. The exposed enamel surfaces were placed in acrylic resin molds, they were covered with nail varnish leaving 2x2 mm window.

Analysis with EDX-SEM was done at baseline, after demineralization and after remineralization. Each specimen was immersed in 10 mL of demineralizing solution for 96 hours to create artificial caries. The solution was prepared using the formula 2.2 mM CaCl<sub>2</sub>, 2.2 mM KH<sub>2</sub>PO<sub>4</sub>, and 0.05 M acetic acid. KOH (1 M) was used to adjust pH to 4.4. Specimens were divided randomly according to the remineralizing agents into four equal groups which were group A: control group (artificial saliva), group B: P11-4 (Curodont repair, Credentis, Windisch, Switzerland) group C: Nano-HA (Nano gate company, Cairo) and group D: CPP-ACPF (GC MI Paste Plus, GC, America).

Remineralization was carried out for a period of 21 days. Remineralizing agents were applied according to manufacture instructions, Curodont repair in group B was applied only once at the beginning of pH cycle while in

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group C and D pastes were applied twice daily for 3 minutes, in group A no remineralizing agent was applied, it follows pH cycling like other groups. The pH cycle model included 3 hours of demineralizing solution with weak acid (pH:4.7) and 21 hours in artificial saliva to simulate the daily acid challenges in oral cavity.

**Results:**

Comparison between study groups after remineralization showed that the highest calcium weight was found in

group B followed by group C and group D then group A (control). (Figure1).The differences were statistically significant in self-assembling peptide and nano-hydroxyapatite compared to control group ( $P < 0.05$ ). Highest Ca/P ratio weight % was found in group C with mean (2.06) followed by group C with mean (2.03) and group D with mean (1.85) then group A with mean (1.49). (Figure2).

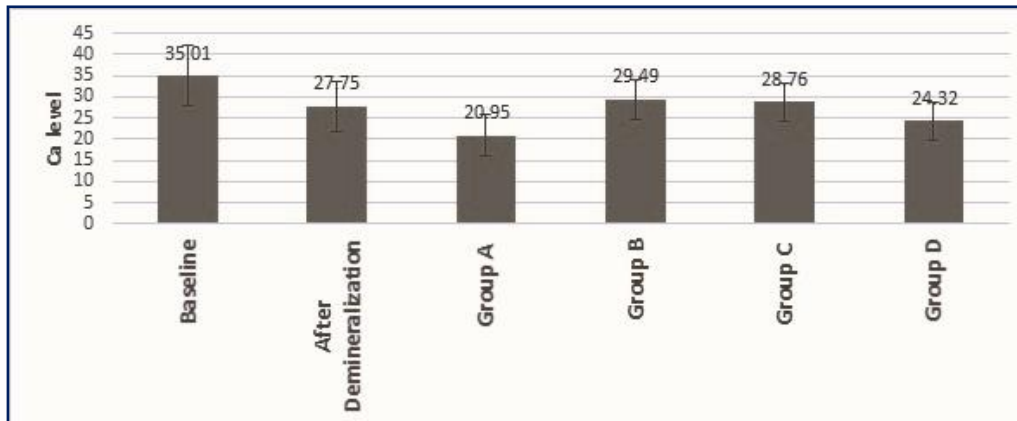


Figure1: Mean Ca level among studied groups at all periods of the study

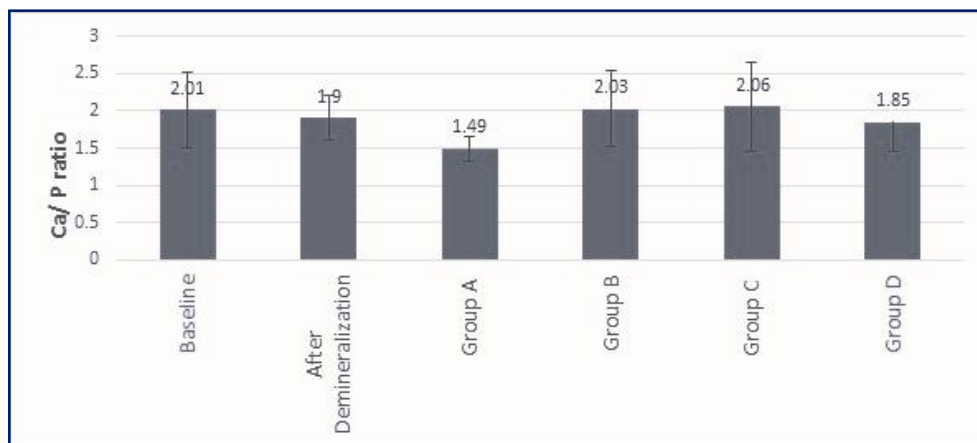


Figure 2: Mean Ca/P ratio among studied groups at all periods of the study

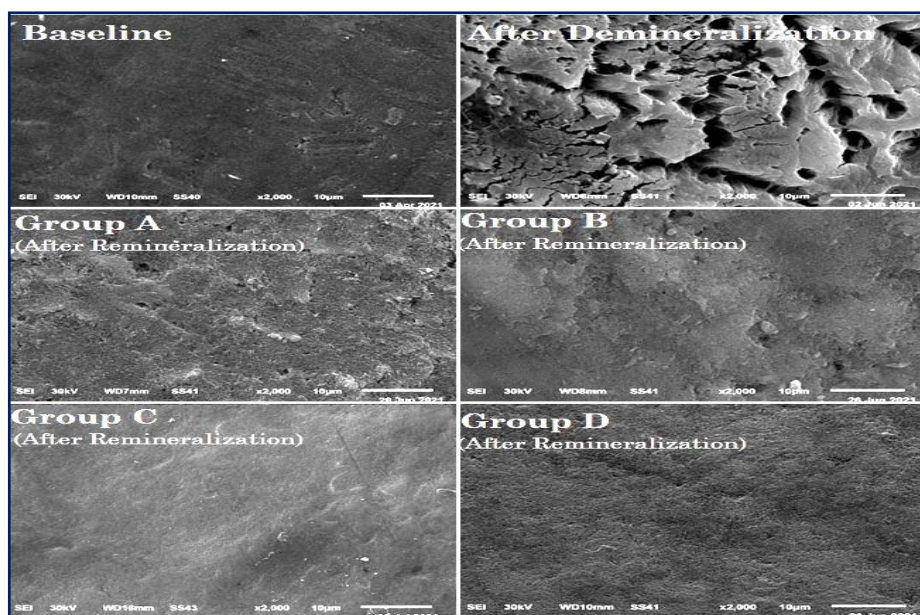


Figure3: SEM comparison of samples at all periods under 2000x.

### Discussion:

Several studies have been focused on remineralization of permanent teeth.<sup>11,12-17</sup> However, trivial researches investigated remineralization effect in primary dentition specially on the materials we used in our study. Therefore in the present study, the aim focused on investigating the remineralizing effect of different agents as Self-assembling peptide, Nano-Hydroxyapatite and CPP-ACPF on primary teeth enamel. In the present study EDX and SEM were used to measure the changes in the mineral content of the carious lesions quantitatively to provide more promising results in remineralization process.

The EDX findings revealed that calcium and phosphorus weight percent (wt%) of all the enamel specimens was significantly reduced from baseline after demineralization regimen and all three-treatment regimen promoted the remineralization of enamel lesions compared to control group after remineralization. The highest mean value was found in self-assembling peptide (P11-4) which had a significant increase in CA and CA/P ratio than control group. The results obtained in this study are in agreement with many studies.<sup>2,18-21</sup>

Nano hydroxyapatite 10% paste group showed the second high remineralizing potential in this study as the CA/P ratio and CA weight percentage increased after remineralization and were significantly higher than control group. In accordance to our result, a study made by Kim et al.<sup>22</sup> reported that higher concentration (10%) of Nano-HA with longer treatment time showed a greater remineralization effect. Also, Huang et al.<sup>23</sup> and Vijayasankari et al.<sup>24</sup> confirmed that 10% Nano-HA has high remineralizing potential. In contrast, the results obtained by Kamath et al.<sup>25</sup> showed no significant difference between the groups. The variation in the result might be due to the concentration of the Nano-HA used in the present study.

CPP-ACPF group showed remineralizing potential in our study which was non-significant compared to control group. A similar result in a study done by Narayana et al.<sup>26</sup> found greater efficacy with bioactive glass than CPP-ACPF. Also, Vijayasankari et al.<sup>24</sup> found that Nano-hydroxyapatite was more effective in remineralizing than CPP-ACP. Kamal et al.<sup>27</sup> and Babaji et al.<sup>28</sup> found that P11-4 had better remineralizing efficacy.

Other studies found that fluoride shows higher remineralizing potential than CPP-ACPF. These results were employed by Lata et al.<sup>29</sup> who stated that CPP-ACPF crème is not as effective as fluoride in remineralizing early enamel caries. This could be attributed to the fact that the study compared a fluoride varnish to a CPP-ACPF tooth crème that might have been easily washed away and so would not have the same contact time as fluoride varnish. On the other hand, other studies showed a significant remineralizing effect of CPP-ACPF like a study done by Thimmaiah et al.<sup>17</sup>. This may be because the study was done on permanent teeth and the pH cycle was 30 days using only artificial saliva.

Based on the results of our study, Self-assembling peptide and Nano-HA showed comparatively more remineralization potential than CPP-ACPF. This result is

similar to a previous study reporting low levels of available fluoride from MI Paste Plus (CPP-ACPF) because this product is highly thick in consistency and does not easily liquefy.<sup>29</sup>

Control group showed the least values of remineralization as saliva fails to initiate the process of increasing the levels of calcium and phosphate delivery compared to the remineralizing regimens applied,<sup>30</sup> which goes in accordance with studies done by Somani et al. and Zhang et al.<sup>31,32</sup>

SEM findings were consistent with the changes in EDX at the different stages of the in vitro study, comparison between groups at all periods was shown at (Figure 3). After application of remineralizing agents, the normal enamel surface smoothness was relatively restored showing a decrease in irregularities and pitting, denoting remineralization. However, enamel remineralization following self-assembling peptide and Nano-HA application showed a marked improvement when compared to specimens treated by CPP-ACPF.<sup>33</sup>

### **Limitations:**

- 1- Remineralization in vitro may be quite different when compared with dynamic complex biological system, which usually occurs in the oral cavity in vivo.
- 2- Lack of studies that have assessed the efficacy of some of these remineralizing agents in primary teeth.

### Conclusion:

1. All tested agents (Self assembling peptide, Nano Hydroxyapatite and CPP-ACPF) had the potential to remineralize artificially induced carious lesions.
2. Self-assembling peptide and Nano hydroxyapatite are considered effective remineralizing agents compared to CPP-ACPF.
3. SEM evaluation confirmed that Self assembling peptide and Nano Hydroxyapatite had the highest delivery vehicle to localize calcium and phosphate at the demineralized enamel surface.

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