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# Effect of adding CPP-ACP into a daily-use toothpaste on remineralization of enamel white spot lesions

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## Abstract

**Objective:** The purpose of this study was to investigate the effect of adding CPP-ACP into a daily-use toothpaste on the remineralization of enamel caries lesions.

**Methods:** Thirty enamel blocks were obtained from bovine incisors. Each specimen was divided into three equal parts. One-third of each block was coated with varnish to serve as a sound control area, while the remaining two-thirds underwent a demineralization process. After demineralization, another one-third of the surface was varnished, leaving only one-third of the enamel to undergo remineralization. The enamel blocks were divided into three groups (n=10), according to the remineralization treatment applied as follows: Group 1: fluoride-containing toothpaste, Group 2: CPP-ACP-containing toothpaste, and Group 3: fluoride- and CPP–ACP–containing toothpaste. Remineralization was assessed through the Vickers microhardness test at various depths (20, 50, 120 and 200 µm). The data were analyzed by ANOVA and LSD test, and $P< 0.05$ was considered statistically significant.

**Results:** There was a significant difference in remineralization efficacy between the groups at the depth of 20 µm ($P<0.001$). Pairwise comparisons revealed that the toothpaste containing both fluoride and CPP-ACP had a significantly greater microhardness than other experimental groups ($P<0.05$). No significant difference was observed between the study groups concerning microhardness at 50, 120 and 200 µm depths ($P>0.05$).

**Conclusions:** CPP-ACP can serve as a suitable alternative to fluoride in daily-use toothpaste for enamel remineralization. The concurrent use of fluoride and CPP-ACP in toothpaste can generate a synergistic remineralizing effect at the enamel surface layer.

**Keywords:** CPP-ACP, Dental enamel, Fluoride, Remineralization, Toothpaste, White spot lesion

## Introduction

Dental caries currently stands as the most prevalent chronic disease on a worldwide scale (1). The prevalence of dental caries varies greatly, ranging from 60 to 90% in primary school children and nearly reaching 100% in adults (2). Tooth enamel, the tough outer layer of tooth crowns, is considered to be the hardest substance in mammalians and remains highly resistant to fractures (3). It is composed of 96% inorganic components (primarily hydroxyapatite crystals) along with 3% water, and 1% organic components (4, 5). The elevated mineral content of enamel, however, renders it vulnerable to demineralization (6). Microbial plaque serves as the main causative factor for dental caries and the development of white spot lesions. Plaque bacteria metabolize dietary carbohydrates, leading to the production of acids as a byproduct. This acid leads to a decrease in the pH of the plaque fluid near the enamel and ultimately results in enamel degradation, dissolution, and decay (7).

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The impact of dairy products such as milk, milk concentrates, and cheese on dental caries has been recognized since the 1980s when it was shown that cheese had a localized anti-cariogenic effect. This protective effect was attributed to the direct chemical interaction of the casein phosphoproteins and the presence of calcium and phosphate in cheese (8, 9). Compounds containing calcium and phosphate, such as casein phospho-peptide amorphous calcium–phosphate (CPP-ACP), have been introduced to neutralize the impact of microbial plaque on the tooth structure (10). CPP-ACP serves to stabilize calcium and phosphate, while also functioning as a buffer to regulate the pH of dental biofilm. This regulation facilitates the maintenance of high levels of calcium and phosphate within the biofilm, consequently reducing demineralization and promoting remineralization (11, 12).

CPP-ACP may be available in various formulations, including topical creams and toothpaste. It can also be integrated into chewing gum (13). A diverse range of dental products containing CPP-ACP, such as GC Tooth Mousse, GC Tooth Mousse Plus, and MI Paste Plus. GC Tooth Mousse includes 10% CPP-ACP, GC Tooth Mousse Plus incorporates 10% CPP-ACP and 0.2% NaF, and MI Paste Plus comprises 10% CPP-ACP combined with 900 ppm fluoride (all produced by GC, Tokyo, Japan).

Several in vitro and in vivo investigations have demonstrated that CPP-ACP exerts a preventive effect on dental caries lesions (10, 14-18). However, other studies suggested that CPP-ACP products may provide limited or negligible benefits in treating caries-affected enamel (19-22).

There is little information on the effects of toothpaste containing a low percentage of CPP-ACP (1% (w/w)), on the remineralization of enamel lesions. If CPP-ACP demonstrates similar abilities in protecting against demineralization as fluoride toothpaste, it could find applications in various areas of preventative dentistry and daily oral care, especially in children, where fluoride-containing toothpastes are often prohibited. The objective of this research was to examine the effect of toothpaste containing CPP-ACP, fluoride, or both CPP-ACP and fluoride on remineralization of enamel caries lesions.

## Materials and Methods

The protocol of the present in vitro study was approved by the Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.DENTISTRY.REC.1399.146).

### *Toothpaste formulations*

CPP-ACP was synthesized in the Dental Materials Research Center of Mashhad University of Medical Sciences following the methodology outlined by Heravi et al. (23).

The experimental daily-use toothpaste used in the present study contained calcium carbonate (40% by weight), carboxymethyl cellulose (3% by weight), glycerin (30% by weight) and distilled water (27% by weight). Sodium fluoride (1100 ppm), CPP-ACP (1% by weight), or both were added to this formulation.

### *Sample preparation*

In this in vitro experimental study, 30 freshly extracted bovine incisors without any carious lesions, cracks, or enamel defects were selected. Following the initial examination, 30 enamel sections were carefully extracted from the tooth crowns using the CNC machine. These sections were then mounted within acrylic resin and meticulously polished using a series of abrasive papers with grit sizes of 600, 800, 1500, 2500, and 5000, respectively.

Subsequently, one-third of each enamel block was varnished so that the underlying area would be sound (control). The remaining two areas were subjected to the demineralization process. For this purpose, the samples were immersed in the demineralization solution comprising 2.2 mM CaCl2, 2.2 mM NaH2PO4, and 0.05 M acetic acid, with the pH adjusted to 4.4 using 1 M KOH (24). The samples were kept in the incubator for 6 days and the solution was changed every 3 days. After the demineralization process, another one-third of the enamel surface was varnished so that only one-third of the surface was available for the remineralization process.

### *Grouping and remineralizing treatments*

The 30 enamel blocks were divided into three distinct groups, each undergoing a specific treatment, as follows:

Group 1: The teeth in group 1 received an experimental daily-use toothpaste containing 1100 ppm of sodium fluoride.

Group 2: The specimens in group 2 received an experimental daily-use toothpaste containing 1% by weight of CPP-ACP.

Group 3: The enamel blocks in group 3 received an experimental daily-use toothpaste containing 1% by

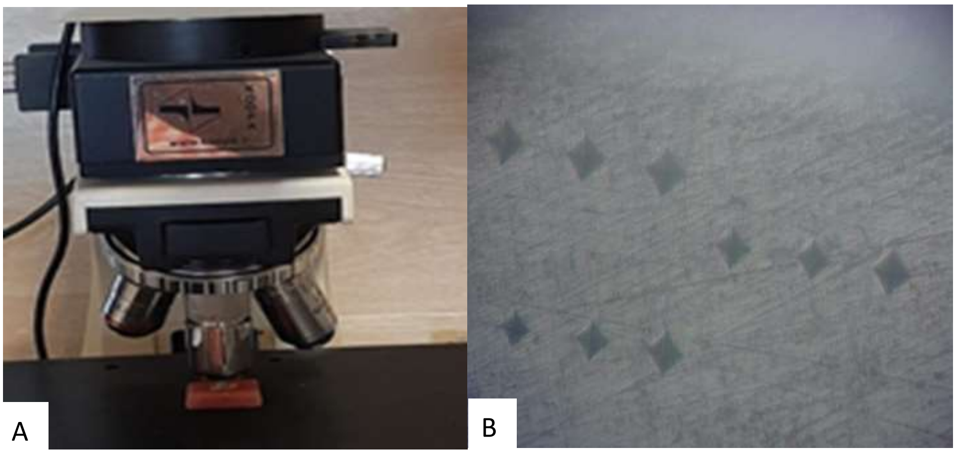


**Figure 1.** A. The Vickers hardness tester used in this study, B. The indentations produced at different depth to measure microhardness of demineralized enamel

weight of CPP-ACP, as well as 1100 ppm of sodium fluoride.

In each of the experimental groups, the designated toothpaste was applied to the third area of the enamel sample for 3 minutes, followed by rinsing with distilled water. Subsequently, the samples were immersed individually in an artificial saliva solution with a pH of 6.5, containing 1 mM calcium ions, 3 mM phosphate ions, and 100 mM sodium fluoride. This process was repeated for 7 days.

### *Microhardness assessment*

Upon completing the remineralization cycle, the samples were embedded in acrylic resin and sectioned along the longitudinal axis, allowing for the observation of cross-sections representing sound, demineralized, and remineralized areas. To quantify the level of remineralization, microhardness was measured at various depths, including 20, 50, 120, and 200 µm from the surface using a Vickers hardness tester (Figure 1). Microhardness values were recorded at each depth for all three regions.

From the collected data, two critical parameters, relative demineralization (rDEM) and relative remineralization (rREM), were calculated as follows:

$$rDEM = \frac{VHN(Dem) - VHN(Sound)}{VHN(Sound)} \times 100$$

$$rREM = \frac{VHN(Rem) - VHN(Sound)}{VHN(Sound)} \times 100$$

VHN: Vickers hardness number
Sound: Sound enamel
Dem: Demineralized region
Rem: Remineralized region

To compare the remineralizing potential between groups, we calculated the 'Remin power' for each group at different depths using their specific rREM and rDEM values, according to the formula mentioned below:

Remin Power = rRem – rDem

### *Sample size calculation and statistical analysis*

The sample size for each group was calculated to be 10, considering $\alpha = 0.05$, $\beta = 0.2$, effect size of 0.50, and a standard deviation of 10. Data were subjected to statistical analysis using SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) and the post hoc least significant difference (LSD) test were employed for data analysis. A P-value less than 0.05 was considered statistically significant.

## Results

Table 1 presents the remineralization power of the study groups at different enamel depths. ANOVA revealed a significant difference in remineralization power among the three groups at a depth of 20 µm ($P<0.001$; Table 1). The post hoc LSD test demonstrated that there was no statistically significant difference between the two groups of toothpastes containing either fluoride or CPP-ACP alone. However, the remineralization power in the toothpaste containing both fluoride and CPP-ACP was significantly higher than in any of the other experimental toothpaste formulations.

There was no significant difference in the remineralization power between the study groups at the depth of 50 µm (P=0.30), 120 µm (P=0.75) and 200 µm

**Table 1.** Comparison of mean and standard deviation (SD) of the remineralization power values at four different depths between the experimental groups

| Group | Definition | 20<br>Mean ± SD | 50<br>Mean ± SD | 120<br>Mean ± SD | 200<br>Mean ± SD |
|---|---|---|---|---|---|
| 1 | Toothpaste containing sodium fluoride | 71.11 ± 13.93[a] | 59.88 ± 18.31 | 46.09 ± 13.99 | 12.79 ± 10.88 |
| 2 | Toothpaste containing CPP-ACP | 70.83 ± 24.54[a] | 62.37 ± 29.19 | 50.44 ± 27.50 | 10.73 ± 11.22 |
| 3 | Toothpaste containing sodium fluoride and CPP-ACP | 104.40 ± 13.80[b] | 77.56 ± 33.98 | 54.83 ± 33.00 | 21.21 ± 16.12 |
| P-value | | <0.001 | 0.30 | 0.75 | 0.17 |

The groups that have been defined by different lowercase letters indicate statistically significant differences at P<0.05.

(P=0.17) (Table 1). A comparison of the remineralization power between groups is illustrated in Figure 2.

## Discussion

This in vitro study investigated the effect of incorporating CPP-ACP into a daily-use toothpaste on the remineralization of bovine enamel lesions, through analyzing the changes in microhardness values. Several research investigations have demonstrated that bovine teeth share comparable physical characteristics and chemical composition with human teeth, making them suitable for in vitro studies (25-27).

The outcomes of this study demonstrated there was no significant difference between the two toothpastes containing fluoride or CPP-ACP when applied separately at any measurement depth. Therefore, it can be assumed that CPP-ACP provides remineralization of white spot lesions similar to fluoride-containing products. A systematic review conducted by Li et al. (15) also reported that CPP-ACP exhibits a long-term remineralizing effect on early caries lesions when compared to a placebo, although this effect does not appear to differ significantly from that of the fluoride treatment. Hidayat et al. (28) investigated the remineralization power of CPP-ACP through enamel microhardness assessment. They applied CPP-ACP as a remineralization material over 21 days and observed enhanced microhardness of demineralized enamel.

CPP-ACP is recognized as a supplier of calcium and phosphate ions in proximity to areas susceptible to demineralization. This treatment is expected to hinder demineralization, promote remineralization, or ultimately achieve both effects (29). The effectiveness of CPP-ACP in enamel remineralization is attributed to casein phosphopeptides, which stabilize calcium phosphates on the tooth surface, resulting in elevated gradients of calcium and phosphate ions around caries lesions (30, 31).

It is important to highlight that a key advantage of CPP-ACP products over fluoride-containing agents is that they do not lead to enamel fluorosis and are safe for ingestion. In contrast, topical fluoride products may pose a health hazard if a significant amount of fluoride is accidentally ingested (27). Therefore, considering the comparable effectiveness of CPP-ACP to fluoride in the remineralization of enamel lesions and taking into account its lower side effects, CPP-ACP can be considered a suitable alternative to fluoride as an anti-caries agent.

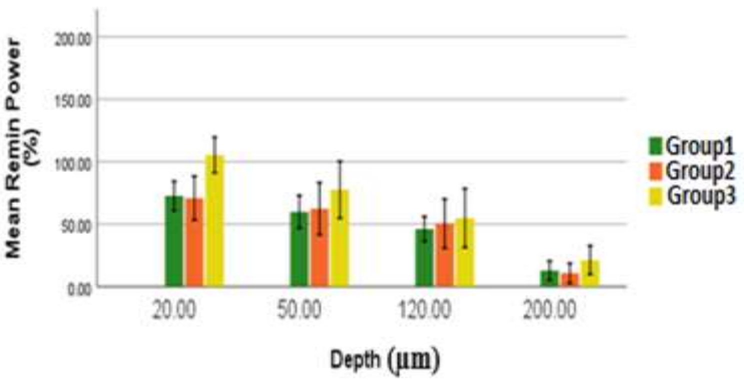


**Figure 2.** Remineralization power values in the experimental groups at four different depths (Group1: Toothpaste containing sodium fluoride; Group 2: Tooth paste containing CPP-ACP; Group 3: Toothpaste containing sodium fluoride and CPP-ACP)

The ability of remineralization in the toothpaste incorporating both fluoride and CPP-ACP was significantly superior to that of the other experimental daily-use toothpaste formulations at a 20 µm depth. Beyond this depth, it was observed that there was no statistically significant difference in remineralization capacity among the groups at different depths (50 µm, 120 µm, and 200 µm).

The findings of this study align with those of Wu et al. (32) who indicated that CPP-ACP tooth mousse could reduce the size and mean grey level of demineralized regions in bovine enamel, and this effect was enhanced by concurrent fluoride use. Mekky et al. (33) displayed enhanced remineralization by adding fluoride to CPP-ACP.

While in vitro studies are valuable for research, they can never perfectly mimic the complex environment of the oral cavity. Therefore, due to the in vitro design of this study, the results cannot be fully extrapolated to clinical conditions.

Further studies are suggested to explore the long-term effects of CPP-ACP toothpaste on enamel remineralization in a clinical setting. Additionally, future research could investigate the optimal concentration of CPP-ACP for maximizing remineralization benefits, while evaluating potential side effects.

## Conclusions

In conclusion, CPP-ACP can serve as a suitable alternative to fluoride in daily-use toothpaste for promoting enamel remineralization. The concurrent use of fluoride and CPP-ACP in toothpaste can produce a synergistic effect at the surface layer of enamel, making it a suitable option in high-risk patients.

## Conflict of interest

There are no conflicts of interest.

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