

8-28-2017

The Effect of Casein Phosphopeptide-amorphous Calcium Fluoride Phosphate on the Remineralization of Artificial Caries Lesions: An In Vitro Study

Ngoc Ngoc Vo Truong Nhu

Department of Pediatric Dentistry, School of Odonto-Stomatology, Hanoi Medical University, Hanoi, Vietnam

Thuy Pham Thi Hong

Faculty of Dentistry, Haiphong Medical and Pharmacy University, Hai Phong, Vietnam

Anh Le Quynh

Department of Basic Dental Sciences, School of Odonto-Stomatology, Hanoi Medical University, Hanoi, Vietnam, quynhanh0191@gmail.com

Son Tong Minh

Department of Basic Dental Sciences, School of Odonto-Stomatology, Hanoi Medical University, Hanoi, Vietnam

Nguyen Thi Thu

Department of Basic Dental Sciences, School of Odonto-Stomatology, Hanoi Medical University, Hanoi, Vietnam

Follow this and additional works at: <https://scholarhub.ui.ac.id/jdi>

Recommended Citation

Nhu, N. N., Hong, T. P., Quynh, A. L., Minh, S. T., & Thu, N. T. The Effect of Casein Phosphopeptide-amorphous Calcium Fluoride Phosphate on the Remineralization of Artificial Caries Lesions: An In Vitro Study. *J Dent Indones.* 2017;24(2): 45-49

This Article is brought to you for free and open access by the Faculty of Dentistry at UI Scholars Hub. It has been accepted for inclusion in Journal of Dentistry Indonesia by an authorized editor of UI Scholars Hub.

ORIGINAL ARTICLE

The Effect of Casein Phosphopeptide-amorphous Calcium Fluoride Phosphate on the Remineralization of Artificial Caries Lesions: An In Vitro Study

Ngoc Vo Truong Nhu¹, Thuy Pham Thi Hong², Anh Le Quynh³, Son Tong Minh³, Phuong Nguyen Thi Thu³

¹Department of Pediatric Dentistry, School of Odonto-Stomatology, Hanoi Medical University, Hanoi, Vietnam

²Faculty of Dentistry, Haiphong Medical and Pharmacy University, Hai Phong, Vietnam

³Department of Basic Dental Sciences, School of Odonto-Stomatology, Hanoi Medical University, Hanoi, Vietnam

Correspondence e-mail to: quynhanh0191@gmail.com

ABSTRACT

The studies on electron microstructure of the effect of the use of products that contain casein phosphopeptide-amorphous calcium fluoride phosphate (CPP-ACPF) on enamel remineralization are still needed. It is important method to observe of the morphological changes of teeth in different conditions. **Objective:** To evaluate the remineralization potential of paste on enamel lesions using scanning electron microscopy (SEM). **Methods:** Sixty enamel specimens were prepared from extracted human premolars. The specimens were placed in a demineralizing solution for four days to produce artificial carious lesions. The specimens were then randomly assigned to two study groups: group A (control group) and group B. Group B was incubated in remineralizing paste (CPP-ACPF) for 30 minutes per day for 10 days. The control group received no intervention with remineralizing paste. All 60 specimens were stored in artificial saliva at 37°C. After remineralization, the samples were observed using SEM. **Results:** The statistical analysis showed a decrease in the lesion area between the demineralized and remineralized samples, but no significant difference was observed in the lesion depth for group B. There was a significant increase observed in both the lesion depth and lesion area for group A ($p = 0.03$). **Conclusion:** The results showed the capacity of CPP-ACPF in supplying calcium and phosphate to the enamel, decreasing the dissolution of the enamel surface and increasing the remineralization of the enamel surface.

Keywords: CPP-ACPF, enamel, fluoride, remineralization, scanning electron microscopy

How to cite this article: Ngoc VTN, Thuy PTH, Anh LQ, Son TM, Phuong NTT. The effect of casein phosphopeptide-amorphous calcium fluoride phosphate on the remineralization of artificial caries lesions: an in vitro study. J Dent Indones. 2017;24(2): 45-49

INTRODUCTION

Tooth caries is a common disease that occurs in all age groups, races, and genders. It is also diagnosed all over the world at a relatively high rate.¹ Fluoride has been vastly studied in the dentistry for a long time. It has commonly been used in the prevention of tooth caries. Recently, fluoride has been combined with Casein phosphopeptide (CPP), a protein found in cow milk, which is able to stably combine with Amorphous Calcium Phosphate to form Casein Phosphopeptide - Amorphous Calcium Phosphate Fluoride (CPP-

ACPF). This compound is proven as effective agent in preventing tooth caries due to the capability of providing fluoride, calcium, and phosphate for the remineralization of enamel.^{2,3}

Research studies on the effectiveness of CPP-ACPF in the remineralization process of caries lesions at an early stage have been conducted in many countries both *in vitro* and *in vivo*. Therefore, the effectiveness of the use of products that contain CPP-ACPF is still unclear due to a lack of studies on its effect on remineralization. Using Scanning Electron Microscopy (SEM), many

authors have identified the benefits of the morphological changes of teeth in different conditions. For instance, the characteristics of demineralized primary enamel subjected to brushing with a dentifrice with or without fluoride.⁶ Due to a lack of clear conclusions, this study was conducted to further evaluate the remineralization potential of caseinphosphopeptide-amorphous calcium fluoride phosphate (CPP-ACPF) paste on enamel lesions using SEM. The results emphasize the importance of using fluoride in combination with phosphate to treat caries at an early stage for permanent teeth; hence, an approach to managing comprehensive caries prevention is proposed to provide an initial healthy dentition for children and to contribute to the findings of in vitro studies in the dentistry field, which is still in need of supporting evidence.

METHODS

Tooth specimen

Sixty permanent premolar teeth were extracted for orthodontic purposes (at the age of 18-25). The extracted teeth were cleaned, removed from all soft tissues, and stored in a 0.1% thymol solution at 37°C. Each tooth should have intact buccal enamel without any fractures, caries, white or brown spots, enamel hypoplasia, dental stains and dental fluorosis.

Sample preparation

The teeth were randomly divided into two groups (A and B) that consisted of 30 teeth. The acid resistant coating was used to cover the entire surface of the enamel crowns of each tooth, leaving only two enamel windows on the buccal surface sized 1x1mm parallel to each other. We used transparent nail polish (Revlon, USA) as the coating agent. The acid resistant coating was applied two times, the second application was done after the first coat had dried. The teeth were soaked in a saline solution at room temperature until use. Before the pH cycle was started the teeth were soaked in 1.2L of demineralization solution (2.2mM CaCl₂, 2.2mM KH₂PO₄, 50mM lactic acid, and 0.02 ppm F⁻. A set pH at 4.4 with a 1M KOH solution) at 37°C for 4 days to create artificial caries lesion.

pH cycle

To mimic the remineralization condition in the oral cavity, the teeth in the Group A was soaked in artificial saliva (Biotene). For the teeth in Group B, the remineralization was done by applying the GC Tooth Mousse (GC Corp., Tokyo, Japan) as the CPP ACPF. For each pH cycle, the teeth were soaked in the demineralization solution twice for 3 hours each. As the rest time, the teeth were exposed in the remineralization condition (in Biotene for Group A and GC Tooth Mousse for Group B) twice for 2 hours each. After the twice cycles were finished, the teeth were soaked in Biotene overnight (15 hours). The cycles were repeated for 10 days.

Image analysis

After the 10 days of pH cycles were finished, the acid resistant coating was removed from all teeth. Each teeth from each group were subjected to SEM analysis. The depth and the area of the enamel structure of the two experimental windows were measured before and after the experiment (intra and between groups). The generated SEM results were analyzed using the Imagepro Plus 6 software.

Data analysis

The data was statistically analyzed using SPSS software version 17. The average value and the standard deviations of the depth and area of the lesions were analyzed by the Kolmogorov-Smirnov. The differences between the pre- and post-pH cycle of in the same group (i.e., between two left and right enamel windows) were identified using the Paired-samples T-test. The differences among specimens in the pre-pH cycle groups and the post-pH cycle groups were investigated using the ANOVA.

RESULTS

Depth analysis

The Table 1 describes the results of the depth analysis using the SEM of the experimental groups. The average of depth before the pH cycle among teeth in Group A and Group B were similar (p=0.2). The average depth after pH cycle treatment among the teeth of the two experimental group were significantly different (p=0.0001). The mean average of depth of the teeth in Group A was higher by approximately 15 points compared to the ones in Group B. The depth of the lesions in the teeth from Group A was significantly increased after pH cycle (p=0.03) compared to depth reduction in the teeth from Group B (p=0.1).

Area analysis

Before treatment, the average area of groups A and B were not significantly different (p=0.1). After

Table 1. Comparison of the depth and area of the experimental areas pre- and post pH cycle among the experimental groups

Measurement	Group A	Group B	P
Depth (µm)			
Pre-treatment	109.61±23.01	111.32±20.39	0.2
Post-treatment	118.99±17.03	103.55±17.32	0.0001
p	0.03	0.1	
Area (µm)			
Pre-treatment	1.13±0.19	1.16±0.21	0.1
Post-treatment	1.23±0.16	1.03±0.21	0.0001
p	0.0001	0.0001	

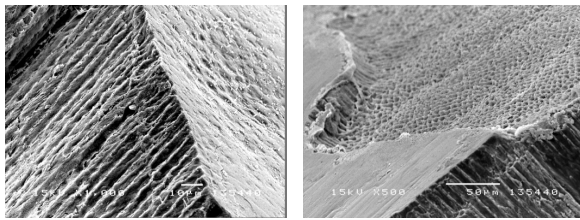


Figure 1. A. The enamel surface before pH cycle. The image shows that some enamel crystals dissolved to form slits on the surface of the enamel. B. The surface of enamel was demineralized further (at a magnification of $\times 500$ and $\times 5000$). A thin surface layer, about $25\mu\text{m}$, was dissolved to expose damaged enamel at the bottom. The picture shows that the pillar body lost more minerals than the spaces between pillars, forming an image of a “honeycomb” surface.

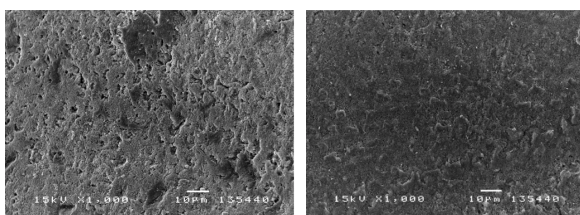


Figure 2. SEM figures of the teeth in Group B at a magnification of $\times 1000$. A. The enamel surface before pH cycle. The enamel crystals were dissolved to reveal the gaps in the enamel surface. B. No obvious gaps in the enamel crystals.

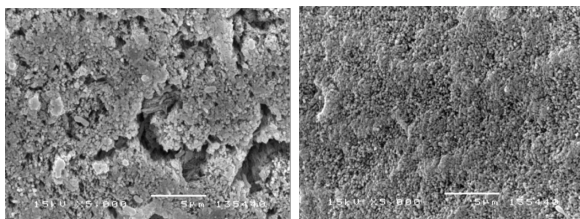


Figure 3. SEM figures of the teeth in Group B at a magnification of $\times 5000$. A. The enamel surface before pH cycle. The enamel crystals were dissolved to reveal the gaps in the enamel surface. B. No obvious gaps in the enamel crystals.

treatment, the average areas of the two groups differed significantly ($p=0.0001$). After the pH cycle, the lesion area of group A tended to increase ($p=0.0001$), but for group B, the lesion depth tended to decrease after the pH cycle ($p=0.0001$) (Table 1).

The teeth in group A were remineralized in artificial saliva, while the teeth in group B were remineralized using GC Tooth Mousse. For group A, the images showed that the pillar bodies lost more minerals than the spaces between pillars, forming an image of a “honeycomb” surface (Figure 1). For group B, there were no obvious gaps in the enamel’s surface at a magnification of $\times 1000$ and $\times 5000$ (Figure 2, 3).

DISCUSSION

The aim of this study was to assess the effects of GC Tooth Mousse Plus on caries lesions at early stages in vitro based on an image analysis performed using SEM. Demineralization was performed in two stages. First, the bacteria metabolized carbohydrates to generate organic acids. The acids diffused into the hard tissues of the tooth via fluid in the crystal network. When the acids reached a sensitive location on the surface of the crystal, carbonate ions replaced phosphate ions, and the acids combined with the carbonate ions, which melted the calcium and phosphate in the solution between the enamel pillars. This phenomenon could only be observed at the molecular level through an electron microscope.^{2,3}

The teeth were soaked in a demineralizing solution that consisted of CaCl_2 , KH_2PO_4 , lactic acid, and fluoride for 4 days at 37°C , and then the second stage of demineralization began, creating artificial caries lesions at an early stage for which the average depth and area were $107\mu\text{m}$ and $1.14\mu\text{m}$, respectively.² The average depth and area of groups A and B had no statistically significant differences prior to treatment. In the demineralizing solution, the concentrations of calcium and phosphate were saturated, therefore causing less damage to the enamel surface and creating the conditions for the formation of lesions in the enamel subsurface. A low concentration of fluoride was added to the solution to protect the enamel surface from acid by creating a fluorapatite layer on the surface, which is similar to natural processes in an oral environment. The images obtained from the SEM showed that the demineralized surface enamel was no longer smooth but became rough, indicating that the enamel crystals were dissolved by lactic acid. The pillar bodies lost more minerals than the spaces between pillars, forming holes in the enamel surface that resembled a “honeycomb” surface. The results of this study are consistent with previous study.⁷ After being soaked in an acid buffer solution ($\text{pH } 4.75$, $0.75\text{mM CaCl}_2 \cdot 2\text{H}_2\text{O}$, $0.45\text{mM KH}_2\text{PO}_4$), the enamel was exposed, and dentine tubules become visible on the surface. The early stage of dentine demineralization seemed to be similar to the erosion pattern of enamel.

Demineralization in the pH cycle lasted for 3 hr, simulating the same process in the oral environment. The remineralizing solution in the enamel windows was applied once per day, simulating a daily tooth protection in oral cavity. The results showed that in group A, there was an increase in both the depth and the average area of lesions after the pH cycle; therefore, in this group, there was no remineralization. This occurred because artificial saliva biotene could not independently remineralize enamel surface lesions. In group B, after the pH cycle, both the lesion depth and area tended to be reduced, but the reduction in the lesion depth had no

statistical significance. This result indicates that group B had more or less remineralization. A comparison of the two groups before the pH cycle revealed that the lesion depth and average area had no significant difference ($p>0.05$); however, after the pH cycle, the lesion depth and the average area of the two groups were significantly different ($p<0.0001$).

The images taken by the SEM also showed that in group A, the level of enamel lesions worsened after the pH cycle. Over time, the images showed hole lesions in the enamel surface. In group B, the slots, which refer to the holes on the surface of lesions after demineralization, were filled after the pH cycle. Thus, saliva biotene could not increase the concentration of calcium ions, phosphate ions, or fluoride ions for the remineralization process that occurs on the surface of the enamel. In contrast, GC Tooth Mousse Plus, which contains CPP-ACPF, was capable of supplying materials for the remineralization of enamel lesions. It is proposed that CPP-ACP was likely a calcium phosphate reservoir because it supported the activities of calcium ions and free phosphate in the surface plaque to help maintain the saturation status during the remineralization of enamel. The remineral ions protect the enamel surface from acid attacks, thus reducing demineralization and promoting enamel remineralization. Reynolds et al. reported that CPP-ACP is similar to bacteria in plaque because it easily attaches to the surface of teeth.³ Hence, the CPP-ACP is deposited with a high concentration of ACP near the surface of the tooth.^{2,3,8}

Reynolds et al.'s results showed that the remineralization process involved in the diffusion of CaHPO_4 , neutral ion pairs of calcium, and phosphate are formed by acid and calcium ions. The phosphate is linked to the hole filled with protein and water on the surface of the tooth. In the body of the tooth, enamel lesions, calcium, and phosphate increase the activities of Ca^{2+} and PO_4^{3-} , which could diffuse the subsurface damage and be deposited in the crystal defect of the demineralized enamel surface.³

The CPP maintained an intensive activity of calcium ions and free phosphate by reserving ACP. This may explain why the calcium solution -CPP was an effective remineralizing solution because it could compensate for the decrease in pH by producing more calcium and phosphate ions, including CaHPO_4 , thus maintaining a high gradient concentration in the lesions.^{3,8} CPP-ACP interacted with fluoride ions to form nano calcium, fluoride, and phosphate. The discovery of a new form of CPP from calcium ions, fluoride, and phosphate was similar to the anti-caries effect of the CPP-ACP nano complex and fluoride. The anti-caries mechanism of fluoride depended on the position of fluoride ions on the tooth's surface. Thus, the formation of fluorapatite

must require the presence of calcium ions and fluoride phosphate. The anti-caries effects of CPP-ACP and fluoride depended on the position of the nano calcium ion, phosphate, and new fluoride on the tooth's surface due to CPP, which utilized calcium, phosphate, and fluoride as useful ions with the exact ratio mole to form fluorapatite.⁸

The study results are consistent with previous studies that showed the efficacy of CPP-ACP in remineralization of the enamel surface.⁹⁻¹² In addition, this study also support that that CPP-ACPF was capable of remineralizing the subsurface of enamel.^{6,13}

CONCLUSION

This in-vitro study confirmed that CPP-ACPF paste was effective in preventing the demineralization of hard tissues and in remineralizing the surface for caries lesions at an early stage for permanent teeth. The anti-caries effects of CPP-ACP and fluoride depended on the position of the nano calcium ion, phosphate, and new fluoride on the tooth's surface due to CPP, which utilized calcium, phosphate, and fluoride as useful ions with the exact ratio mole to form fluorapatite.

REFERENCES

1. Loc Giang Do, Spencer AJ, Roberts-Thomson KF, Hai Dinh Trinh, Thuy Thanh Nguyen. Oral health status of Vietnamese children: findings from the National Oral Health Survey of Vietnam 1999. *Asia Pac J Public Health*. 2011;23(2):217-27.
2. Featherstone JD, Doméjean S. The role of remineralizing and anticaries agents in caries management. *Adv Dent Res*. 2012 ;24(2):28-31.
3. Reynolds EC. Casein phosphopeptide-amorphous calcium phosphate: the scientific evidence. *Adv Dent Res*. 2009;21(1):25-9.
4. Patil N, Choudhari S, Kulkarni S, Joshi SR. Comparative evaluation of remineralizing potential of three agents on artificially demineralized human enamel: An in vitro study. *J Conserv Dent*. 2013;16(2):116-20.
5. Kumar VL, Itthagarun A, King NM. The effect of casein phosphopeptide-amorphous calcium phosphate on remineralization of artificial caries-like lesions: an in vitro study. *Aust Dent J*. 2008;53(1):34-40.
6. Neves Ade A, Castro Rde A, Coutinho ET, Primo LG. Microstructural analysis of demineralized primary enamel after in vitro toothbrushing. *Pesqui Odontol Bras*. 2002;16(2):137-43.
7. Maki O, Kanako Y. Effect of CPP-ACP paste on tooth mineralization: an FE-SEM study. *J Oral Sci*. 2007;49(2):115-20.

8. Azarpazhooh A, Limeback H. Clinical efficacy of casein derivatives: A systematic review of the literature. *J Am Dent Assoc.* 2008;139(7): 915-24.
9. Hegde MN, Moany A. Remineralization of enamel subsurface lesions with casein phosphopeptide-amorphous calcium phosphate: A quantitative energy dispersive X-ray analysis using scanning electron microscopy: An in vitro study. *J Conserv Dent.* 2012;15(1):61-7.
10. Ferrazzano GF, Amato I, Cantile T, Sangianantoni G, Ingenito A. In vivo remineralising effect of GC tooth mousse on early dental enamel lesions: SEM analysis. *Int Dent J.* 2011;61(4):210-6.
11. Lata S, Varghese NO, Varughese JM. Remineralization potential of fluoride and amorphous calcium phosphate-casein phosphopeptide on enamel lesions: An in vitro comparative evaluation. *J Conserv Dent.* 2010;13(1):42-6.
12. Jayarajan J, Janardhanam P, Jayakumar P, Depika. Efficacy of CPP – ACP and CPP – ACPF on enamel remineralisation – An in vitro study using scanning electron microscope and DIAGNOdent. *Indian J Dent Res.* 2011;22(1):77-82.
13. Reynolds EC. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. *J Dent Res.* 1997;76(9):1587-95.

(Received November 2, 2016; Accepted July 7, 2017)