Journal of Dental Biomaterials
Shiraz University of Medical Sciences, Shiraz, Iran

Journal of Dental Biomaterials. 2016;3(2)

Original Article

Concentration of Calcium, Phosphate and Fluoride Ions in Microbial Plaque and Saliva after Using CPP-ACP Paste in 6-9 year-old Children

Poureslami H[a], Hoseinifar Ra[b], Hoseinifar Re[c], Sharifi H[d], Poureslami P[e]

[a]Oral and Dental Diseases Research Center AND Kerman Social Determinants on Oral Health Research Center AND Department of Pediatric Dentistry, School of Dentistry, Kerman University of Medical Sciences, Kerman, Iran
[b]Department of Operative Dentistry, School of Dentistry, Kerman University of Medical Science. Kerman, Iran
[c]Department of Pediatric Dentistry, School of Dentistry, Kerman University of Medical Sciences, Kerman, Iran
[d]Regional Knowledge Hub, and WHO Collaborating Centre for HIV Surveillance, Kerman University of Medical Sciences, Kerman, Iran
[e]School of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran

ARTICLE INFO

Article History:
Received: 22 February 2016
Accepted: 25 May 2016

Key words:
Dental Plaque
Fluoride
Calcium

Corresponding Author:
Reihaneh Hoseinifar
Department of Pediatric Dentistry, School of Dentistry, Kerman University of Medical Sciences, Kerman, Iran
Email: reihanehosseinifar@gmail.com
Tel: +98-9133415534

Abstract

***Statement of Problem*:** Dental caries is one of the most common chronic diseases in children. The balance between demineralization and remineralization of the decayed teeth depends on the calcium and phosphate content of the tooth surface. Therefore, if a product such as casein phospho peptides - amorphous calcium phosphate (CPP-ACP) which can significantly increase the availability of calcium and phosphate in the plaque and saliva should have an anti-caries protective effect.

Objectives: The purpose of this study was to evaluate the concentration of calcium, phosphate and fluoride in the plaque and saliva of children before and after applying the CPP-ACP paste.

Materials and Methods: A total of 25 children aged between 6-9 years were selected for this clinical trial study**.** At first, 1 ml of unstimulated saliva was collected and then 1 mg of the plaque sample was collected from the buccal surfaces of the two first primary molars on the upper jaw. In the next step, CPP-ACP paste (GC Corp, Japan) was applied on the tooth surfaces and then the plaque and saliva sampling was performed after 60 minutes. The amount of calcium ions was measured by Ion meter instrument (Metrohm Co, Swiss) and the amounts of phosphate and fluoride ions were measured by Ion Chromatography instrument (Metrohm Co, Swiss). Data were analyzed using paired *t*-test at a $p < 0.05$ level of significance.

Results: There were statistically significant differences in the calcium and phosphate concentration of the saliva and plaque before and after applying the CPP-ACP paste. There were also statistically significant differences in the fluoride levels of the plaque before and after applying the CPP-ACP paste. However, there were no statistically significant differences in the fluoride levels of the saliva before and after applying the CPP-ACP paste**.**

Conclusions: In this study, the use of the CPP-ACP paste significantly increased the fluoride levels of the plaque and the calcium and phosphate levels of both saliva and plaque. Hence, CPP-ACP paste can facilitate the remineralization of tooth surfaces and is useful for protecting the primary teeth.

Cite this article as: Poureslami H, Hoseinifar Ra, Hoseinifar Re, Sharifi H, Poureslami P. Concentration of Calcium, Phosphate and Fluoride Ions in Microbial Plaque and Saliva after Using CPP-ACP Paste in 6-9 year-old Children. J Dent Biomater, 2016;3(2): 214-219.

Introduction

Dental caries is the most common chronic infectious disease in children [1]. A recent study [2] reported that the prevalence of caries in 6-9 year old Iranian children was 89-90%, with a mean dmft score ranging about 3.3-4.8. While the mean dmft scores among children in Saudi Arabia (2010), Brazil (1996), China (2002), Palestine (2007) and Philippines (1998) were 5,3.9,4.5,6.5 and 4.6 respectively [3-6].

Dental caries can affect the children's quality of life. In most small children (less than 2 years old), dental decay lead to reduced weight gain and reduced growth [7]. Impaired speech development and reduced self-esteem have been reported to be associated with early tooth loss caused by dental caries [8].

The current focus of researches conducted in this field is the development of non-invasive treatment approaches [9], and casein phospho peptides-amorphous calcium phosphate(CPP-ACP) has been introduced by Eric Reynold and coworkers as a therapeutic approach to enhance the remineralization [10]. CPP-ACP is a bioactive substance basically made from milk products and is composed of two parts: casein phospho peptides (CPP) and amorphous calcium phosphate (ACP) [11].

CPP has a great potential to stabilize calcium and phosphate in "nanocluster" forms of ions in solution and to remarkably enhance the level of calcium and phosphate in dental plaque [11]. CPP can bind to the biofilm, enamel and soft tissues, thereby delivering the calcium and phosphate ions exactly to the sites where they are needed. The free calcium and phosphate ions move out of the CPP, enter the enamel prisms, and restore the apatite crystals [12].

CPP-ACP has been shown to have anticariogenic properties in laboratory, and also in animal and human in situ experiments. The anticariogenic mechanism of CPP-ACP is incorporating ACP in dental plaque, which buffers the free calcium and phosphate ions and creates a state of supersaturated level of calcium and phosphate regarding the tooth enamel; this reduces the enamel demineralization and promotes remineralization [11].

CPP-ACP is useful in the treatment of dental caries, white spot lesions, hypocalcified enamel, dentin hypersensitivity, mild fluorosis, and erosion. CPP-ACP can also prevent demineralization around the brackets and other orthodontic appliances and facilitate a normal post-eruptive maturation process [11].

CPP-ACP properties such as inhibition of demineralization, enhanced remineralization and its bacteriostatic/bactericidal effect are similar to the single most useful anticaries agent, fluoride, while CPP-ACP demonstrates none of the adverse effects of fluoride such as fluorosis at moderate doses and toxicity at higher doses [13,14].

Sufficient amounts of calcium and phosphate ions must be available for net remineralization to occur, but this process is normally restricted by the amount of available calcium and phosphate [15]. Therefore, if a product such as CPP-ACP can significantly increase the availability of calcium and phosphate in plaque, it should have an anti-caries protective effect by decreasing demineralization, enhancing remineralization, or probably a combination of both [13].

In the previous studies, only the calcium and phosphate concentrations of the saliva after chewing the CPP-ACP containing gum were evaluated, and there is no report about the fluoride concentrations of the saliva and plaque and also calcium and phosphate concentrations of the plaque after applying the CPP-ACP paste. Therefore, the aim of this study was to evaluate the concentration of calcium, phosphate and fluoride in the plaque and saliva of children aged between 6-9 years before and after applying the CPP-ACP paste.

Materials and Methods

A total of 25 students aged between 6-9 years, with good oral hygiene, no active caries and no systemic disease, and resident in a charity educational center in Kerman/Iran, was selected to take part in this clinical trial study.

Ethical approval for this study was obtained from the Ethics Committee of Kerman University of Medical Sciences (IR.Kmu.REC.1394.594). After taking the informed consent, the bacterial plaque was removed using a slow-speed hand piece and a soft rubber cup without using any other substances. The subjects were asked to avoid brushing teeth, using dental floss or fluoride and other medical substances for 48 hours, and then sampling of the plaque and saliva was performed as follows:

Collecting and preparing saliva samples

Saliva sampling was done prior to dental plaque sampling. The subjects were instructed to expectorate a minimum of 1 ml of unstimulated saliva over 5 minutes into a sterile plastic container and then the samples were immediately transferred to the laboratory. Then, 1 ml of salivary samples was transferred to the coded micro-tubes using a sampler. For the preparation of the samples, 900 µl of the saliva sample was mixed with 100 µl of 1 M $HClO_4$ solution and after 2 hours, 100 µl of 90% TISAB III solution (JENWAY, England) was added to the mixture; then, the samples were centrifuged at 12000 rpm for three minutes.

Collecting and preparing plaque samples

Before plaque sampling, the subjects were asked to swallow to remove any pooled saliva. Using sterile applicators (Kerr, USA), we collected the plaque samples from the buccal surfaces of the two first primary molars on the upper jaw and then 1 mg of the sample was transferred into a coded microtube containing 1.5 ml of mineral oil. In order to increase the sensitivity of the measurements, plaque samples were weighted with a digital scale with the sensitivity of 10^{-4} g. All micro-tubes were centrifuged at 12000 rpm for 5 minutes. Subsequently, the collected samples were mixed with 200 µl of 1 M $HClO_{4\ solution}$ and diluted with 1800 µl of TISAB III solution for stability and recording ionic power. All plaque and saliva samples were filtered by a 0.2µ filter. The preparation of the plaque and saliva samples was performed according to the previously described technique by Vogel *et al*. [16].

The amount of calcium ions of the plaque and saliva samples was measured by Ion meter instrument (Metrohm Co, Swiss) and a calcium specific electrode, and the amount of phosphate and fluoride ions was measured by Ion Chromatography instrument (Metrohm Co. Swiss) using their specific columns.

After the first stage of the research, the children followed their usual oral health habits and diet for 14 days. Subsequently, bacterial plaque from the surfaces of the teeth was removed and they were asked to avoid brushing the teeth, using dental floss, fluoride and other medical substances for 48 h. Subsequently, the CPP-ACP paste (GC Corp, Japan) was applied by a sterile swap on the buccal surfaces of the two first primary molars of the upper jaw and then plaque and saliva sampling was performed after 60 minutes. The subjects were asked to avoid eating, drinking and rinsing of the mouth in the period between the application of CPP-ACP paste and collection of the samples. The procedures were repeated similar to the first stage. Data were analyzed by SPSS (v.13.5) software package using paired *t*-test at a $p < 0.05$ level of significance.

Results

The mean calcium, phosphate and fluoride concentrations of the plaque and saliva before and after applying CPP-ACP paste are shown in Table 1. The results showed that the use of CPP-ACP paste significantly increased the calcium, phosphate and fluoride concentrations of the plaque. ($p < 0.001$,

Table 1: The mean calcium, phosphate and fluoride concentrations of the plaque and saliva before and after application of the CPP-ACP paste

Group			Mean	Std.Deviation	*p* value
Saliva	Calcium(µg/ml)	Control	19.04	10.1	< 0.001
		CPP-ACP	43.87	24.7	
	Fluoride(ppm)	Control	0.65	0.15	0.512
		CPP-ACP	0.68	0.15	
	phosphate(µg/ml)	Control	0.33	0.06	< 0.001
		CPP-ACP	0.92	0.26	
plaque	calcium(µg/ml)	Control	22.17	9.1	< 0.001
		CPP-ACP	48.8	23.2	
	Fluoride(ppm)	Control	0.79	0.15	0.029
		CPP-ACP	0.98	0.3	
	phosphate(µg/ml)	Control	0.65	0.26	< 0.001
		CPP-ACP	1.88	0.56	

SD: Standard deviation

$p < 0.001$, $p = 0.029$, respectively).

The results also showed that the use of the CPP-ACP paste significantly increased the calcium and phosphate concentrations of the saliva. ($p < 0.001$, $p < 0.001$, respectively). However, the use of the CPP-ACP paste did not significantly increase the saliva fluoride levels ($p = 0.512$).

Discussion

Several studies have reported an inverse association of plaque Calcium, Phosphate levels and caries experience [17,18].

The results of this study showed that the use of CPP-ACP paste significantly increased the saliva and plaque calcium and phosphate levels. This is consistent with the results of the previous studies.

For example, Kakatkar *et al.* [19] evaluated the impact of CPP–ACP containing gum on the salivary concentration of calcium and phosphate and indicated that CPP-ACP containing chewing gum increased the calcium concentration of the saliva significantly; however, a significant decrease was observed in the phosphate concentration of the saliva for up to 1 hour after chewing the gum, as compared to the baseline. They concluded that this pseudo decrease in the salivary concentration of phosphate could be attributed to the increase in the salivary flow rate after chewing the CPP–ACP containing gum [19].

Reynolds *et al*. [20] evaluated the retention ability of CPP-ACP in supragingival plaque when delivered in a chewing gum or mouth rinse and reported that CPP-ACP-containing mouth rinse significantly increased the level of calcium and inorganic phosphate in supragingival plaque and demonstrated that CPP-ACP could still be detected on the plaque 3 hours after chewing the gum. Moreover, electron microscopic analysis of the supragingival plaque samples indicated that the CPP-ACP was bound to the intercellular matrix of the plaque and on the surface of the bacterial cells [20], confirming the work of Rose who demonstrated that CPP-ACP binds tightly to Streptococcus mutans and model plaque[13,14]. In the mentioned studies, only the calcium and phosphate concentrations of the plaque and saliva after chewing the CPP-ACP containing gum were evaluated, while in the current study the fluoride concentrations of the saliva and plaque were also examined. Moreover, in the current study the studied subjects were children.

In the present study, the mean concentrations of calcium and phosphate (in both the plaque and saliva) after applying CPP-ACP paste were more than two times and three times (respectively) as much as that in the baseline. Fluoride ions can promote the remineralization of previously demineralized enamel if adequate amounts of plaque or salivary calcium and phosphate ions are available when the fluoride is applied (For every two fluoride ions, six phosphate ions and ten calcium ions are needed to form one unit cell of fluorapatite ($Ca_{10}(PO_4)_6F_2$)) [21]. Additionally, it is reported that high extracellular free calcium concentrations may have bacteriostatic or even bactericidal effects. Hence, it is possible that due to the maintenance of high free calcium, CPP-ACP may have an additional anti-plaque effect [13,22]. These results highlight the importance of the availability of plaque or salivary calcium and phosphate ions.

In a physiologic condition, the buffering potential of the saliva and its content of ions retains the PH of the oral cavity close to the saturation state [23]. However, due to various reasons, this balance may move towards demineralization of tooth structure. Srinivasan *et al*. [24] observed that no remineralization occurred in the eroded enamel which was immersed in the saliva compared to groups treated with CPP-ACP and CPP-ACPF. In many cases, saliva cannot shift towards remineralization by itself because of the presence of mature bacterial plaques or active caries; therefore, remineralizing agents are necessary for shifting towards remineralization [24].

Morgan *et al.* [25] evaluated the effect of CPP-ACP containing gum on the approximal caries. In this two year study, the children were assigned to either a test (CPP–ACP containing gum) or control (sugar-free gum without CPP-ACP) groups. All subjects received accepted preventive procedures, including fluoridated dentifrice, fluoridated water and access to professional care. But the CPP-ACP gum (the tested group) significantly slowed progression and enhanced remineralization of approximal caries relative to the control gum after 24-months. Moreover, the analyses of the sections of remineralized enamel by the CPP-ACP demonstrated that the deposited mineral was hydroxyapatite with higher Calcium: Phosphate ratio than normal hydroxyapatite; therefore, the remineralized enamel was more resistant to acid challenge [25].

In the current study, the use of the CPP-ACP paste significantly increased the fluoride concentrations of the plaque. Some researchers believe that the accumulation of fluoride on the plaque depends on the presence of calcium ions. They attributed this fact to the role of salivary calcium in the bacterial cell wall bonding of fluoride[26,27]. Therefore in the current study, the significant increase in the fluoride concentration of the plaque is related to the availability of high amounts of calcium ions. Reynolds *et al.* [21] also evaluated the ability of CPP-ACP to enhance the incorporation of fluoride into the plaque, indicating that the addition of 2% CPP-ACP to the 450-ppm-fluoride rinse increased the fluoride incorporation into supragingival plaque significantly, where the fluoride concentration of the plaque was over double that obtained with the fluoride rinse alone. CPP-ACP also increased the fluoride incorporation into subsurface enamel and substantially increased the enamel subsurface remineralization compared with fluoride alone. Moreover, the dentifrice containing 2% CPP-ACP plus 1100-ppm-F' produced a more homogenous remineralization throughout the body of the lesion [28].

Fluoride was introduced into dentistry over 70 years ago [29]. Fluoride ions play several significant roles in caries-prevention; these include the formation of fluorapatite crystals, which are more acid resistant than hydroxyapatite, interference with ionic bonding during plaque and pellicle formation, the enhancement of remineralization and the inhibition of the microbial growth and metabolism [30].

Conclusions

In this study, the use of the CPP-ACP paste significantly increased the fluoride levels of the plaque and calcium and phosphate levels of both saliva and plaque. Hence, CPP-ACP paste can facilitate the remineralization of tooth surfaces and is useful for protecting the primary teeth, especially when oral hygiene is not desirable.

Refrences

1. Shammukha G, Jethmalani SB, Basavaraj N, *et al.* Evaluation of changes in salivary concentration calcium by CPP-ACP containing chewing gum–A clinical trial. Int J Adv Res Oral Sci. 2012;1:1-7.
2. Pakshir HR. Oral health in Iran. Int Dent J. 2004;54:367-372.
3. Al Agili DE. A systematic review of population-based dental caries studies among children in Saudi Arabia. Saudi Dent J. 2013;25:3-11.
4. Bagramian RA, Garcia-Godoy F, Volpe AR. The global increase in dental caries. A pending public health crisis. Am J Dent. 2009;22:3-8.
5. Wang HY, Petersen PE, Bian JY, *et al*. The second national survey of oral health status of children and adults in China. Int Dent J .2002;4:283-290.
6. Bajali M, Sgan-Cohen HD, Abdulgani E, *et al*. Oral health status of Palestinian children in the West Bank-Preliminary Data. Middle East J Oral Health. 2007;1:10-12.
7. Petersen PE, Estupinan-Day S, Ndiaye C. WHO's action for continuous improvement in oral health. Bull World Health Organ. 2005;83:642.
8. Çolak H, Dülgergil ÇT, Dalli M, *et al.* Early childhood caries update: A review of causes, diagnoses, and treatments. J Nat Sci Biol Med. 2013;4:29-38.
9. Rezvani MB, Karimi M, Rasoolzade RA, *et al*. Comparing the Effects of Whey Extract and Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) on Enamel Microhardness. J Dent. 2015;16:49-53.
10. Walsh LJ. Contemporary technologies for remineralization therapies: A review. Int Dent SA. 2009;11:6-16.
11. Reema SD, Lahiri PK, Roy SS. Review of casein phosphopeptides-amorphous calcium phosphate. Chin J Dent Res. 2014;17:7-14.
12. Jayarajan J, Janardhanam P, Jayakumar P, *et al*. Efficacy of CPP-ACP and CPP-ACPF on enamel remineralization-An in vitro study using scanning electron microscope and DIAGNOdent®. Indian J Dent Res. 2011;22:77-82.
13. Rose RK. Binding characteristics of Streptococcus mutans for calcium and casein phosphopeptide. Caries Res. 2000;34:427-431.
14. Rose RK. Effects of an anticariogenic casein phosphopeptide on calcium diffusion in streptococcal model dental plaques. Arch Oral Biol. 2000;45:569-575.
15. Hegde MN, Devadiga D, Jemsily PA. Comparative evaluation of effect of acidic beverage on enamel surface pre-treated with various remineralizing agents: An In vitro study. J Conserv Dent. 2012;15:351-356.
16. Vogel G, Zhang Z, Chow L, *et al.* Changes in lactate and other ions in plaque and saliva after a fluoride rinse and subsequent sucrose administration. Caries Res. 2002;36:44-52.
17. Moreno E, Margolis H. Composition of human plaque fluid. J Dent Res. 1988;67:1181-1189.

18. Shaw L, Murray J, Burchell C, *et al.* Calcium and phosphorus content of plaque and saliva in relation to dental caries. Caries Res. 1983;17:543-548.
19. Kakatkar G, Nagarajappa R, Bhat N, *et al.* Evaluation of Salivary Calcium and Phosphorous Concentration Before and After Chewing CPP-ACP Containing Chewing Gum. Acta stomatologica Croatica. 2012;46:117-125.
20. Reynolds E, Cai F, Shen P, *et al.* Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. J Dent Res. 2003;82:206-211.
21. Reynolds E. Calcium phosphate-based remineralization systems: scientific evidence? Aust Dent J. 2008;53:268-273.
22. Kobayashi H, Van Brunt J, Harold FM. ATP-linked calcium transport in cells and membrane vesicles of Streptococcus faecalis. J Biol Chem. 1978;253:2085-2092.
23. Heshmat H, Banava S, Abdian H, *et al.* Effects of Casein Phosphopeptide amorphous Calcium Phosphate and casein phosphopeptide amorphous Calcium Phosphate Fluoride on alterations of dental plaque pH following sucrose consumption. J Islamic Dent Assoc IRAN. 2014;26:274-279.
24. Srinivasan N, Kavitha M, Loganathan S. Comparison of the remineralization potential of CPP–ACP and CPP–ACP with 900ppm fluoride on eroded human enamel: An in situ study. Arch Oral Biol. 2010;55:541-544.
25. Morgan M, Adams G, Bailey D, *et al*. The anticariogenic effect of sugar-free gum containing CPP-ACP nanocomplexes on approximal caries determined using digital bitewing radiography. Caries Res. 2008;42:171-184.
26. Cochrane N, Cai F, Huq N, *et al.* New approaches to enhanced remineralization of tooth enamel. J Dent Res. 2010;89:1187-1197.
27. Kato K, Nakagaki H, Arai K, *et al*. The influence of salivary variables on fluoride retention in dental plaque exposed to a mineral-enriching solution. Caries Res. 2002;36:58-63.
28. Reynolds E, Cai F, Cochrane N, *et al.* Fluoride and casein phosphopeptide-amorphous calcium phosphate. J Dent Res. 2008;87:344-348.
29. Buzalaf MA, Pessan JP, Honório HM, *et al.* Mechanisms of action of fluoride for caries control. Mongor Oral Sci. 2011;22:97-114.
30. Niessen L, Gibson G. Oral health for a lifetime: Preventive strategies for the older adult. Quintessence Int. 1997;28:626-630.