Effect of casein phosphopeptide-amorphous calcium phosphate on the microhardness and microscopic features of the sound enamel and initial caries-like lesion of permanent teeth, compared to fluoridated agents

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ABSTRACT

Background: Casein phosphopeptide-amorphous calcium phosphate derived from the milk protein casein. The aims of this study were to investigate the efficacy of CPP-ACP on the microhardness of the sound and artificially caries-like lesion of outer enamel surface in comparison to sodium fluoride 0.05%, stannous fluoride 0.4%, and de-ionized water.

Materials and methods: Sixty five maxillary first premolars, two teeth were used for sound and caries- like lesion enamel ground section preparation, while other teeth were randomly divided into two groups, A and B. Group A was consisted of 42 teeth were randomly assigned to five study groups and one control group. After production of initial caries-like lesion of outer enamel surface, the teeth were treated by the selected agents for four minutes separately (CPP-ACP, CPP-ACP+NaF, CPP-ACP+SnF_2, NaF 0.05%, and SnF_2 0.4%). While Group B consisted of 21 teeth were randomly divided to three study groups, teeth treated with (CPP-ACP, CPP- ACP+NaF, CPP- ACP+SnF_2) before demineralization by pH cycling procedure.

Results: In both groups showed that agents of study groups were statistically highly significant in elevation of the microhardness values, CPP-ACP+NaF caused highest change in the microhardness (207.21%) for Group A and (19.22%) for Group B, while the lowest change with SnF_2 0.4% (74.32%) for Group A and CPP-ACP (8.2%) for Group B.

Conclusions: CPP-ACP agents were effective in remineralization of the outer enamel caries-like lesions and the higher remineralizing potential when applied with fluoridated agents; which was reflected by increase in enamel microhardness values.

Key words: Demineralization, caries, pH-cycling, CPP-ACP, remineralization.

Abbreviations and acronyms: CPP-ACP = casein phosphopeptide-amorphous calcium phosphate; NaF = sodium fluoride; SnF_2 = stannous fluoride. (J Bagh Coll Dentistry 2012; 24(4):114-120).

INTRODUCTION

Over the last few decades, fluoride in various forms has been proven to reduce caries in both the primary and permanent dentitions when used in a variety of ways. The widespread reduction in dental caries worldwide is largely due to the extensive use of fluoride in the form of toothpastes and mouthrinses [(1,2)]. The efficacy of these oral care products has been attributed to their ability to incorporate fluoride ions into plaque and enamel, since several investigators have suggested an inverse relationship between plaque fluoride levels and caries [(3)].

The anticariogenic properties of milk and milk products, such as cheese, have been studied previously in animal models [(4,5)]. This activity has been attributed to the direct chemical effects of phosphoprotein casein and calcium phosphate components in cheese [(6,7)]. In recent years casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) have also been demonstrated to have anticariogenic properties in both laboratory animal and human in situ experiments [(8,9)].

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Casein phosphopeptides (CPP) are peptides derived from the milk protein casein that are complexed with calcium and phosphate. In this complex, the CPP maintains the calcium and phosphate in an amorphous form called amorphous calcium phosphate (ACP). The CPP binds to surfaces such as plaque and soft tissue providing a reservoir of bioavailable calcium and phosphate, at the surface of the tooth without nucleation and precipitation [(10)].

In a previous studies conducted to detect changes in remineralization and demineralization status of tooth structure, it was concluded that the inorganic components contained in high concentrations in CPP-ACP enhanced remineralization [(11)]. In this study, the effect of CPP-ACP agentswere evaluated on the sound and initial carious enamel surface using Vickers microhardness test (VHT).

MATERIALS AND METHODS

The sample: Teeth samples in this study consisted of 65 maxillary first premolars extracted from 11-14 years old patients, referred for Orthodontic Department, College of Dentistry, university of Baghdad. Teeth were extracted a traumatically as much as possible, washed with

de-ionized water, and then each tooth was wiped with acetone to remove any debris, then stored in 20 ml of de-ionized water to which 0.1% thymol was added to prevent microbial growth. Then teeth samples were kept in refrigerator at 4˚C until use.

Enamel surface preparation: A position of circular window of 6mm in diameter on the buccal surface of each tooth was standardized using orthodontic ruler, an imaginary line was drawn from the tip of buccal cusp to the cervical line and another one between the most prominent curvature of mesial and distal surfaces, therefore the middle area of each surface was identified. Then an adhesive tape circle of 6mm diameter was cut and burnished on the buccal surface of the tooth using burnisher, after that an acid resistant nail varnish was used to paint the surfaces of the tooth, the adhesive tape was removed leaving a window on the buccal surface; Figure (1). Teeth were adapted in an acrylic model (the size of this model was 30 × 27 mm) using a red wax. The grit paper (grit 400) was placed in special manual device; Figure (2).The window of each tooth was ground and polished ten times in one direction. This procedure allowed a flat surface of each tooth for microhardness testing [13].

Caries like Lesion Induction in Enamel Specimens: This procedure was conducted by preparation of demineralizing and remineralizing solutions and adjustment of pH [14]. The demineralizing solution, which contained 0.075 M/L acetic acid, 1 mM/L calcium chloride, and 2 mM/L potassium phosphate had the pH adjusted to 4.3, while the remineralizing solution, which contained 150 mM/L potassium chloride, 1.5 mM/L calcium nitrate, and 0.9 mM/L potassium phosphate had a pH of 7.

The pH cycling procedure: Each cycle involved 6 hours of demineralization with 17 hours of remineralization, the procedure was repeated for a period of ten days, one time each day [14].

Groups design: Two teeth were used for ground section preparation of sound and demineralised enamel (After pH cycling), while other (63) teeth were divided into two groups**:** ***Group A:*** consisted of (42) teeth, they were randomly divided into five study groups and one control group, and each group consisted of six teeth for microhardness test and one tooth for microscopical examination. Then they were subjected to pH cycling procedure.

Enamel microhardness was measured initially for normal enamel and after induction of caries lesion by pH cycling procedure, and finally after treated by the selected agents (CPP-ACP, CPP-ACP+NaF, CPP-ACP+SnF_2, NaF (0.02%), and SnF_2 (0.4%). The microhardness measurement was done by Vickers microhardness device in the Department of Mechanical Engineering, University of Baghdad at a load of 500 gm for 30 seconds.

Group B: consisted of (21) teeth, they were randomly divided into three study groups, and each group consisted of six teeth for microhardness test and one tooth for microscopical examination. Enamel microhardness was measured initially for normal enamel, and after treated with the selected agents (CPP-ACP, CPP-ACP+NaF, CPP-ACP+SnF_2), and finally after induction of caries lesion by pH cycling procedure.

Enamel ground section preparation for microscopical examination: Sections were made bucco- lingually. The preparation of these sections was achieved in the Department of Oral Diagnosis, College of Dentistry, Baghdad University, using Minitom device. Each enamel longitudinal section with the thickness of 0.5 mm was stacked on the middle of glass slide using Canada balsam [15]. Enamel slabs were examined under light microscope (500X). Microscopic examination involving sound enamel surface, after induction of caries lesion by pH cycling procedure and following treatment with the selected agents.

RESULTS

Group A showed that casein phosphopeptide-amorphous calcium phosphate plus fluoridated agents, sodium fluoride and stannous fluoride were statistically highly significant in elevation of the microhardness values of demineralised enamel surface (table 1). However, none of the mentioned agents able to increase the elevation of the microhardness values from sound enamel, which is statistically significant. In Group B, there was also an elevation of the microhardness values from sound enamel, which is statistically significant (table 2). Casein phosphopeptide-amorphous calcium phosphate with sodium fluoride caused highest change in the microhardness (207.21%) for Group A, and (19.22%) for Group B, while the lowest change with stannous fluoride 0.4% (74.32%) for Group A and casein phosphopeptide-amorphous calcium phosphate (8.2%) for Group B (figures 3 and 4).

Figure 5 (a) shows the microscopic characteristics of the sound enamel surface. The highly mineralized enamel surface is demonstrated as clear and intact enamel surface. Microscopic features of the enamel surface after pH cycling procedure is explained in Figure 5(b). A considerable loss of minerals is shown by clear

area of demineralization in the outermost layers of enamel surface. Zone of remineralization in enamel can be seen after treatment with CPP-ACP, CPP-ACP+NaF, CPP- ACP+SnF_2, sodium fluoride, and stannous fluoride, the microscopic changes shown in Figures 5 (c), (d), (e), (f), and (g) respectively.

Figures 6 (a), (b), and (c) show the microscopic characteristics of the enamel surface after demineralization of enamel surface treated with CPP-ACP, CPP-ACP+NaF and CPP-ACP+SnF_2. A considerable loss of minerals is shown by small area of demineralization in the outermost layers with presence of deep layer of mineralized enamel.

DISCUSSION

The remineralizing potential of CPP-ACP has been shown in animal studies [16], in *in vitro* studies [17-20], and in *in vivo* studies [8,21]. It has been proposed that the anticariogenic mechanism of CPP-ACP is due to localization of ACP at the tooth surface which then buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to the enamel, so depressing demineralization and promoting remineralization [22,23].

In group A, after treatment of enamel samples with CPP-ACP agents, sodium fluoride 0.05% and stannous fluoride 0.4%, there was an elevation in the microhardness value, this elevation was statistically highly significant for all. This may be an indication of incorporation of ions that decrease porosity and increase the microhardness of demineralized enamel; such an observation was not seen for samples treated with de-ionized water. This finally also confirmed by microscopic examination, which showed a zone of remineralization in enamel and dentin after treatment with all CPP-ACP agents, sodium fluoride 0.05% and stannous fluoride 0.4% [24]. An interesting result recorded in this study was the higher microhardness values for CPP-ACP+NaF and less for CPP-ACP+SnF_2 and lesser for CPP-ACP. This can be explained by the ability of CPP-ACP to interact with fluoride ions to produce an additive effect through the formation of a stabilized amorphous calcium fluoride phosphate phase [25].

In group B, After treatment of enamel with CPP-ACP agents, there was an elevation in the microhardness values, these elevations, statistically, were significant for all CPP-ACP agents. This can be attributed to CPP-ACP agents contained more bio-available calcium and phosphate, for this reason, CPP-ACP agents can supply minerals into the enamel surfaces, in addition to the effect of fluoride. Statistically, a highly significant reduction was found in the microhardness of enamel surface after pH cycling as an indication of enamel demineralization and initiation of carious lesion which was also confirmed by microscopic examination. A microscopic examination showed a zone of remineralization in enamel with no differentiated zones of enamel caries after demineralization of all CPP-ACP agents. Although remineralization occurred but none of the used agents were capable of increasing enamel microhardness to approximate the original values. This may be due to the time of application used in this experiment. Teeth were treated for four minutes daily for seven days period. Increase the time of treatments to weeks rather than one week may increase the microhardness values; however, this may need to be investigated by further studies.

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Table 1: Microhardness (Mean* and Standard Deviation) of Enamel Surfaces Treated by CPP-ACP, CPP-ACP+ NaF, and CPP-ACP+ SnF_2 after pH Cycling (Group A)

Groups	Casein phosphopeptides-amorphous calcium phosphate		Casein phosphopeptides-amorphous calcium phosphate+sodium fluoride		Casein phosphopeptides-amorphous calcium phosphate+stannous fluoride	
Variables	Mean	±S.D	Mean	±S.D	Mean	±S.D
Sound enamel	248.83	9.66	298.44	18.37	262.72	11.47
Demineralization	51.34	14.32	60.74	12.29	51.66	13.08
Remineralization	122.73	12.94	186.61	8.8	155.69	10.43
ANOVA	F= 386.314		F= 449.859		F= 487.150	
	P= 0.000		P= 0.000		P= 0.000	
	df= 2		df= 2		df= 2	

*(VHN)

Table 2: Microhardness (Mean* and Standard Deviation) of Enamel Surfaces Treated by CPP-ACP, CPP-ACP+ NaF, and CPP-ACP+ SnF_2 before pH Cycling (Group B)

Groups	Casein phosphopeptides-amorphous calcium phosphate		Casein phosphopeptides-amorphous calcium phosphate+sodium fluoride		Casein phosphopeptides-amorphous calcium phosphate+stannous fluoride	
Variables	Mean	±S.D	Mean	±S.D	Mean	±S.D
Sound enamel	260.108	11.117	242.775	10.15	274.552	11.29
Treated enamel	281.441	11.469	289.44	9.69	306.387	10.83
Demineralization	70.417	3.163	91.7	3.97	83.683	6.44
ANOVA	F= 916.175		F= 903.666		F= 903.666	
	P= 0.000		P= 0.000		P= 0.000	
	df= 2		df= 2		df= 2	

*(VHN)

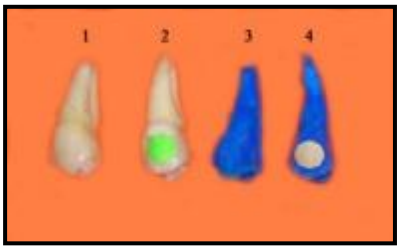

Figure 1: Preparation of window on buccal surface

Figure 2: Manual device for flattening

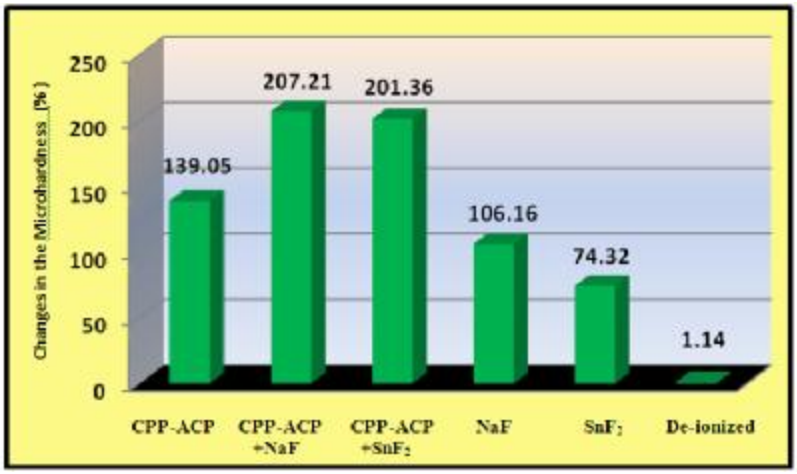

Figure 3: Changes in the Microhardness Values (%) after Treatment with the Selected Agents (Group A)

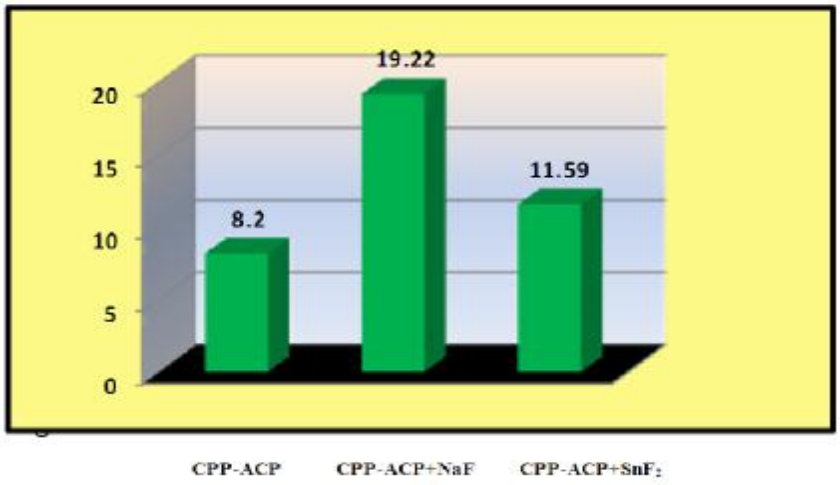

Figure 4: Changes in the Microhardness (%) Values after Treatment with the Selected Agents (Group B)

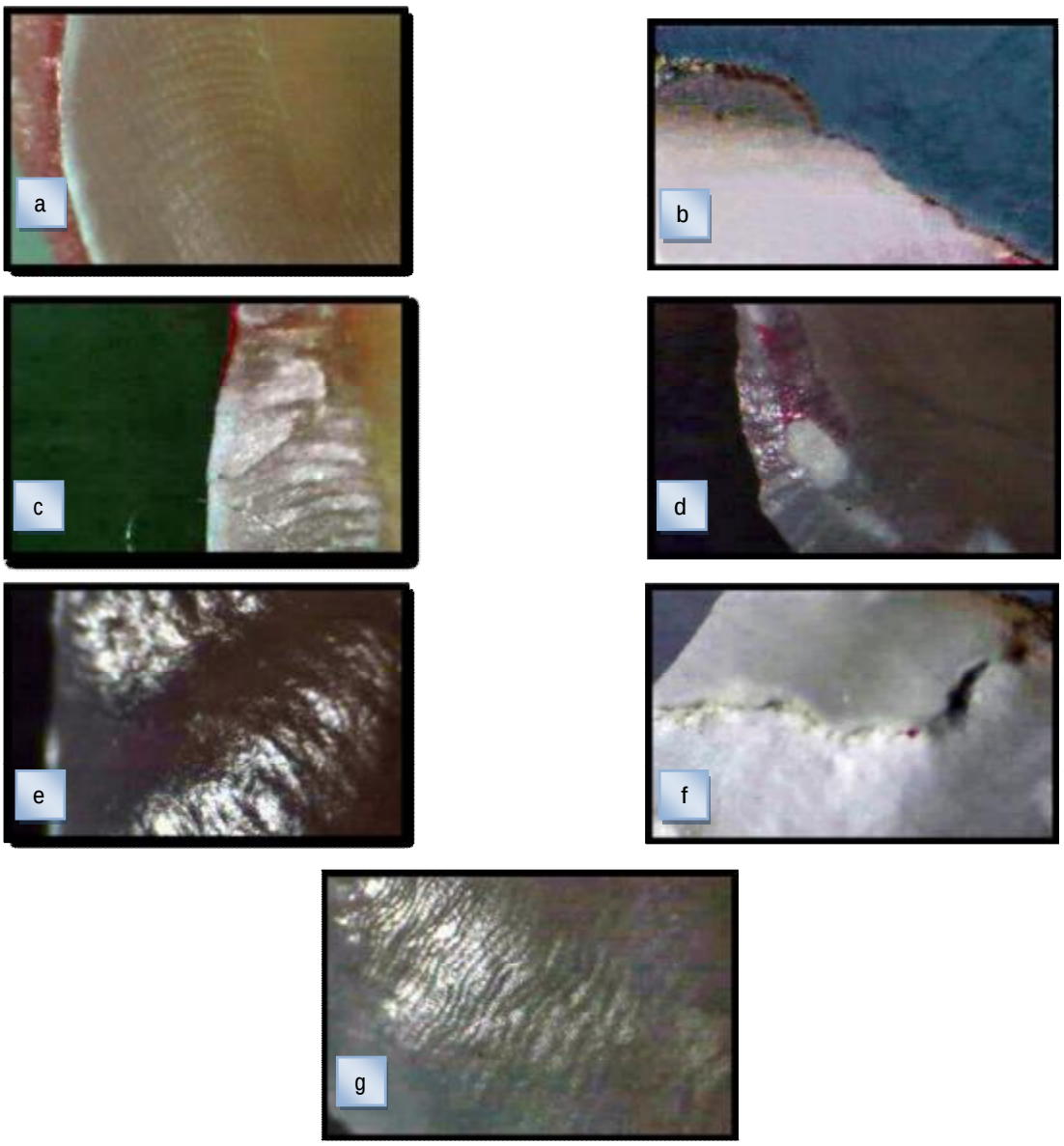

Figure 5: Ground section (500X) of enamel surface:
(a) Sound enamel surface
(b) Demineralised enamel surface, and remineralized enamel surface after treatment with (c) Casein phosphopeptide-amorphous calcium phosphate
(d) Casein phosphopeptide-amorphous calcium phosphate plus sodium fluoride
(e) Casein phosphopeptide-amorphous calcium phosphate plus stannous fluoride
(f) Sodium fluoride
(g) Stannous fluoride (Group A)

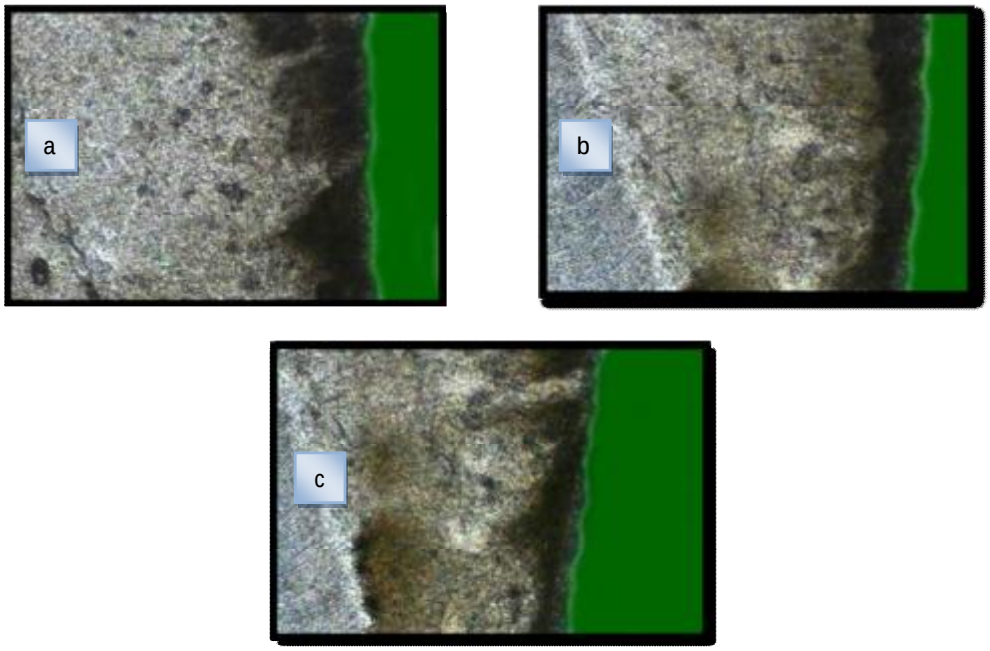

Figure 6: Demineralised enamel surface treated with:
(a) Casein phosphopeptide-amorphous calcium phosphate
(b) Casein phosphopeptide-amorphous calcium phosphate plus sodium fluoride
(c) Casein phosphopeptide-amorphous calcium phosphate plus stannous fluoride (Group B)