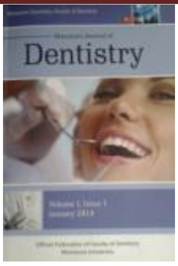
Mansoura Journal of Dentistry 2014;1(3):90-95.



Impact of Topically Applied PEPSI Drink on Enamel and Dentin Surfaces with and without Application of "Casein Phosphopeptide–Amorphous Calcium Phosphate with Fluoride"

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Abstract:

Objectives: An increase in consumption of acidic products has resulted in an increase in the incidence of dental erosion[1]. A newer concept for remineralization is the use of milk and milk products due to the presence of casein. Casein phosphopeptides amorphous calcium phosphate with flouride (CPP-ACPF) is one such agent that has been proposed to have remineralization properties.

Methods: This study determined the impact of PEPSI, a carbonated drink, on enamel and dentin with and without application of CPP-ACPF. Sixty sound posterior permanent teeth that had been freshly extracted for orthodontic reasons were selected after clinical and radiographic examinations. Each tooth was longitudinally sectioned bucoo-lingulally and mesio-distally into four sections perpendicular to the long axis of the root. The cervical one third of each specimen was the selected area for the current study. The specimens were divided into 3 groups; 80 specimens for each, each divided into 4 subgroups. Group I: artificial saliva (control group). Group II: specimens were subjected to PEPSI drinks. Group III: CPP-ACPF paste applied to the specimens then subjected to PEPSI drinks. All the specimens were analyzed using Environmental scanning electron microscopy (ESEM) after 1, 2, 3 and 4 weeks. The results of ESEM were statistically analyzed using two-way ANOVA test followed by LSD post-hoc test.

Results: The application of CPP-ACPF paste was significantly significant decrease both the enamel surface roughness and the diameter of dentinal tubules enhancing the remineralization potential of enamel and dentin.

Conclusions: CPP-ACPF paste is effective in prevention enamel and dentin erosions and a promising material for remineralization.

keywords: *PEPSI drink, enamel, dentin, casein phosphopeptide–amorphous calcium phosphate with fluoride..*

Introduction

In the oral environment, tooth structure undergoes continuous demineralization and remineralization. If this balance is disrupted, demineralization will progress leading to deterioration of the tooth structure [1-3]. It has been reported that the consumption of soft drinks like PEPSI might lead to dental erosion [4]. It is not always practical to eliminate the cause of tooth erosion in individuals, so it is desirable to develop other effective preventive strategies to manage tooth erosion [5].

There is a scope for agents which may be used to enhance remineralization. Recently, dairy products (milk, milk concentrates and cheeses) have received a lot of attention for their anticariogenic effect in animal and human in situ caries models. The protective effect of dairy products is possibly best attributed to the phosphoprotein casein and calcium phosphate contents [1].

CPP-ACP has been proposed to have remineralization properties[2]. At the same time, the fluoride ion has been shown to reduce the speed of demineralization and enhance the reproduction of hydroxyapatite crystals [6]. CPP-ACPF paste which is a new material introduced recently to the market combining both CPP-ACP and fluoride.

Calcium (Ca) and phosphate (P) are essential components of enamel and dentin and form highly insoluble complexes, i.e. forms a crystalline structure at neutral PH. However, in the presence of CPP they remain soluble and biologically available [7]. The CPP keeps the calcium and phosphate in an amorphous, non-crystalline state. In this amorphous state, calcium and phosphate can enter the tooth surface and enhance remineralization [8].

Materials and methods

Sixty sound posterior teeth that had been freshly extracted for orthodontic reasons were used in this study after clinical and radiographic examinations. They were collected from healthy individuals according to a protocol approved by the Ethical Committee of Mansoura University. They were stored in 0.5% sodium azide solution at 4ºC to prevent any fungal or bacterial growth and used within 1 month after extraction. The criteria for tooth selection included the enamel surface being unaffected by any pretreatment chemical agents, no evidence of white spot lesions, enamel cracks or caries and presence of no restorations.

Each tooth was longitudinally sectioned bucoo-lingually and mesio-distally into four sections perpendicular to the long axis of the root using a high speed diamond tipped disc. Four specimens were prepared from each tooth. (60 teeth, 4×60= 240 specimens, total).

No polishing was carried out for the enamel surface to avoid removal of the outer prismless enamel in order to simulate the enamel condition in the oral cavity. Dentin surfaces of specimens were polished using 3M soflex paper discs. Polishing was carried out for 15 seconds using a low speed contra angle hand piece with water cooling. After polishing, the specimens were thoroughly rinsed with distilled water then etched for 20 seconds using 37% phosphoric acid gel (Alpha-etch 37) (Nova DFL) to remove the smear layer created on dentin surfaces after cutting.

The specimens were divided into 3 groups (N=240) and each group divided into 4 subgroups; 80 specimens each, examined after 1, 2, 3 and 4 weeks. Group I: specimens were stored in artificial saliva at room temperature as control group. Group II: specimens were subjected to pH cycling regimen twice daily. One complete pH cycle consisted of immersing in 10 ml of a freshly opened PEPSI drink in a separate container for 5 minutes at room temperature then in artificial saliva. Group III: Thin film of CPP-ACPF paste was rubbed with a gloved finger to the enamel and dentin surfaces and left for 3 minutes, then delicately removed with cotton tips and immersed in artificial saliva for 30 minutes. After that the specimens were subjected to PEPSI drink in a separate container for 5 minutes at room temperature then in artificial saliva. These steps were done twice daily.

The entire surfaces of treated enamel and dentin were examined under ESEM however, only photomicrographs of the cervical areas were taken. Two-way ANOVA statistical test was performed to analyze data obtained from enamel surface roughness and the values of dentinal tubules diameter. This was followed by LSD post-hoc test for treatment groups and time periods multiple comparisons.

Results

Micro-morphological observations of the enamel surfaces with ESEM

Group I (control group): Enamel scanning showed normal homogenous smooth enamel morphology with some craks appeared that were probably due to excessive home tooth brushing. (Fig. 1A, B) Group II (PEPSI group): Erosion of enamel surface was more obvious with the longer exposure period after 4 weeks which represented by significant increase in the enamel surface roughness in comparisons with group I. (Fig. 1C, D). Group III: ESEM image showed a relatively smooth enamel surface with no evidence of porosities or irregularities. It was interesting to detect the least surface roughness after 4 weeks (Fig. 1D,E).

Micro-morphological observations of the dentin specimens with ESEM

Group I (control group): showed normal appearance of dentin (Fig. 2A). Group II (PEPSI group): the dentinal tubules diameters were increased, widening to the extent of becoming connected with the neighboring lateral branches. Peritubular dentin was disappeared and mostly this was obvious after 4 weeks (Fig. 2B). Group III: showed different levels of possible effect of the demineralization/remineralization process taking place in dentin surface. After 1 week, the diameter of dentinal tubules did not seem to undergo modification. After 2 weeks, only a few dentinal tubules were partially occluded by small deposits while after 3 and 4 weeks, more dentinal tubules were seen occluded due to formation of different sized mineral particles. Some of the dentinal tubules were completely blocked (Fig. 2C & D). The diameter of dentinal tubules has decreased; this suggested the protective effect of the CPP-ACPF paste on dentin that decreased dentin demineralization.

Statistical analysis of ESEM results

Enamel surface roughness

Descriptive statistics of enamel surface roughness showed the highest mean value was after 4 weeks in group II, while the lowest after 4 weeks in group III (Fig. 3). Statistical analysis comparisons using two-way ANOVA among different groups and the interaction between groups at different time periods showed high significance among all variables ($p < 0.001$) and it was border line significance among different time periods ($p = 0.055$) (Table 1)

The diameter of dentinal tubules

Descriptive statistics of the diameter of the dentinal tubules showed the highest mean value was after 4 weeks in group II, while the lowest one was after 4 weeks in group III (Fig. 4).

Statistical analysis comparisons using two-way ANOVA among different groups and the interaction among groups and different time periods showed a highly significant difference ($P < 0.001$) while it was non-significant in-between different time periods ($P = 0.09$) (Table 1).

Discussion

In spite of the enamel remineralizing potential of saliva, by itself it fails to initiate the process of increasing the levels of calcium and phosphate [9]. Remineralization has been a major area of investigation. Prevention of initiation and interruption in progression of erosive lesions are the desirable modes of erosion management as Padmini et al. [10], mentioned. Reynolds [11] stated that Ca and P ions must first penetrate the surface layer of enamel, to bring about deposition of minerals through the body of the lesion, which confirms the reason for the CPP supported metastable calcium phosphate solutions of being such efficient remineralizing solutions.

The present *in vitro* study was undertaken to determine the impact of PEPSI, a carbonated drink, on enamel and dentin with and without application of CPP-ACPF paste. PEPSI was used in this study as one of the most commonly consumed acidic beverages. Parry et al. [12] reported that removal of gas from the PEPSI drink may increase its pH and decrease its potential of dissolving hydroxyapatite. Therefore, PEPSI containers were hermetically sealed. Artificial saliva was selected as storage medium so as to simulate the oral environment as described by Singh et al. [13].

The enamel and dentin specimens were obtained from the cervical portion of the crown in an attempt to standardize specimens and the location of an acid attack would be similar to that occurring *in vivo*.

In the present study, the enamel and dentin in group II (PEPSI group) were subjected to pH cycling regimen twice a day in attempt to simulate the real life situation and the dynamics of pH changes occurring in the oral cavity. It is well known that pH cycling regimens are an effective way to simulate the oral environment *in vitro* [14]. The morphological analysis carried out with ESEM of the enamel specimens at different subgroups, showed that changes in surface roughness were evident with prolonged exposure time to PEPSI drinks thus indicated enamel demineralization. These findings come in accordance with Chandna et al. [15] who stated that for enamel demineralization to take place the pH of enamel surface must fall below pH 5.5; acidic soft drinks have a low pH value which decreases the enamel pH below this critical value. In addition, Sauro et al. [16] mentioned the fact that acidic drinks acts as chelator; binding minerals such as Ca.

The dentin specimens of the group II, showed increase in the dentinal tubule diameters with prolonged exposure time to PEPSI drinks and highly significant variations (*P <* *0.001*) when compared with group I. These observations come in accordance with Brajdić [17] who reported that prolonged exposure to phosphoric acid led easily to complete dissolving of dentin peritubular cuff, thus increased the dentinal tubules diameter and decreased the area of intertubular dentin.

In the present study, the specimens of group III were subjected to pH cycling regimen after the application of the CPP-ACPF paste. Reynolds & Walsh [18] suggested that CPP-ACP molecules need an acid challenge to be activated. The morphological analysis carried out with ESEM of enamel specimens showed decrease in enamel surface roughness. The comparisons between group III and group II showed highly significant decrease ($P < 0.001$) in the enamel surface roughness in group III. These observations indicate the protective effect of CPP-ACPF paste against enamel erosions.

These results were in agreement with Badr & Ibrahim [19] who stated that the protective effect of CPP-ACP lies in the fact that it provides a reservoir of bioavailable Ca and P that maintain supersaturated levels, thus inhibits enamel demineralization. Through their multiple phosphoseryl sequences, CPP binds to ACP in metastable solution preventing dissolution of Ca and P ions as explained by Reynolds and Walsh [18].

The dentin specimens of the group III, showed decrease in the diameter of dentinal tubules. The comparisons between group III and group II showed highly significant decrease (*P < 0.001*) in the diameter of the dentinal tubules. These results were in agreement with the study done by Al Zraikat et al. [20] who demonstrated that the release of CPP-ACP and fluoride from CPP-ACP containing glass ionomer cement was associated with enhanced protection of the adjacent dentin during acid challenge *in vitro*.

Although the present study could not simulate the complex oral environment, it showed the potential of CPP-ACPF paste to reverse the harmful effect of PEPSI drinks on enamel and dentin surfaces.

Conclusion

Taking in consideration the limitations of the present *in vitro* study, we can conclude that the application of CPP-ACPF paste was effective in preventing enamel and dentin erosions produced by PEPSI drinks and enhanced the remineralization potential. CPP-ACPF paste is a promising remineralizing material thus; dental practitioners should recommend CPP-ACPF to their patients, particularly children, as well as adults who are at high risk of dental erosions.

Acknowledgment

The authors are grateful to Dr. Mohammed El-Awady Grawish, *Professor of Oral Biology, Faculty of Dentistry, Mansoura Univ.*, for his contribution to this research performing the statistical works.

Table 1: Two-way ANOVA test between-subjects and its statistical significant.

Dependent Variable: Enamel surface roughness					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Groups	106974.640	2	53487.320	334.943	.000
Periods	1260.496	3	420.165	2.631	.055
Groups * Periods	29288.396	6	4881.399	30.568	.000
Dependent Variable: Diameter of dentinal tubules					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Groups	141.900	2	70.950	421.348	.000
Periods	1.129	3	.376	2.234	.090
Groups * Periods	15.270	6	2.545	15.114	.000

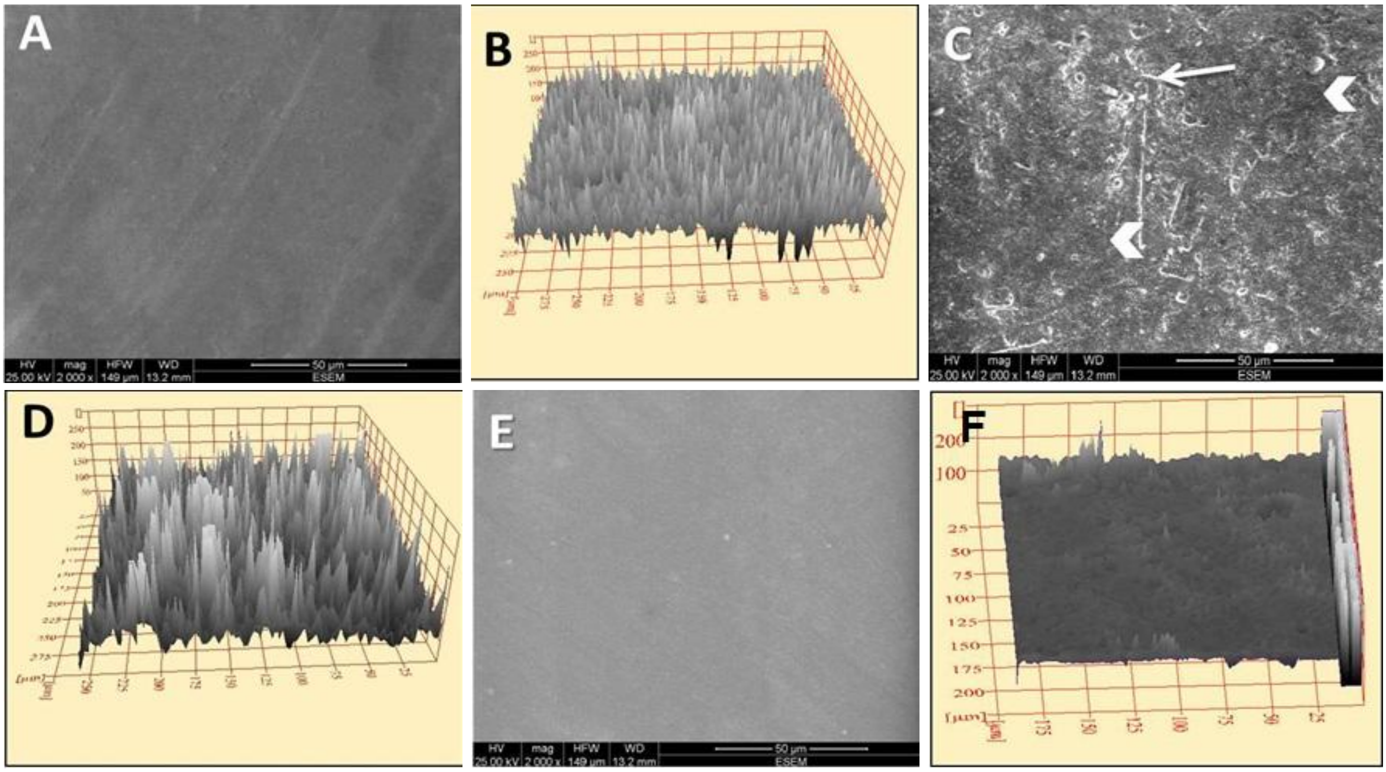

Figure1: ESEM photomicrograph (×2000) of the enamel after 4 weeks. A (group I): shows show untreated enamel surfaces kept in artificial saliva. Aprismatic layer remnants were present on the intact surface. C (group II): shows areas of porosities created by PEPSI that are clearly evident on enamel surface (*white arrow*) and depression areas *(head arrows).* E (group III): shows a relatively smooth enamel surface. (Note: no evidence of porosities or irregularities). B, D and F: ESEM histogram shows enamel surface roughness after 4 weeks (B: group I; D: group II; F: group III). The least surface roughness is shown in F.

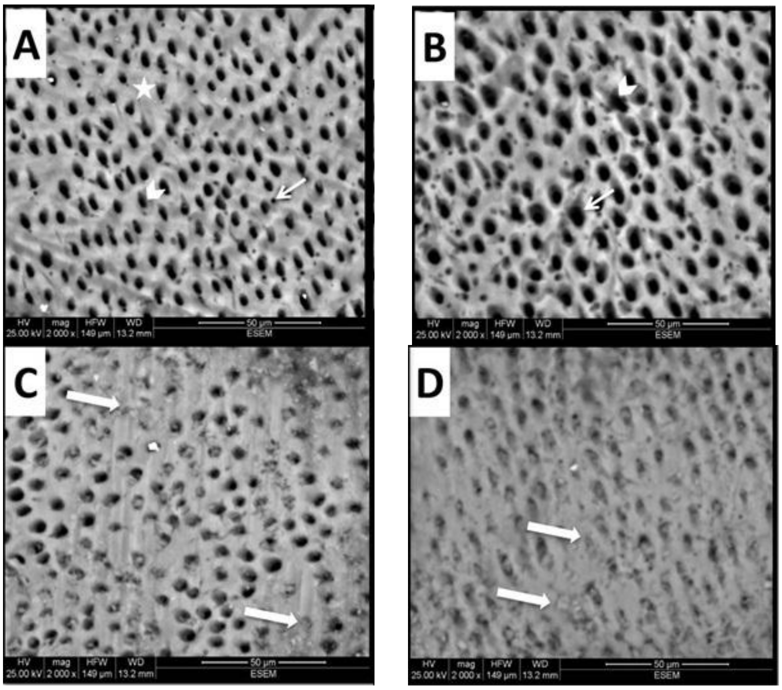

Figure 2: ESEM photomicrographs (×2000) show dentin surface. A, Group I (after 4 weeks): shows Normal appearance of dentin, dentinal tubules orifices exhibit uniformly circular outline encircled by normal peritubular dentin *(head arrow)*, normal homogenous appearance of intertubular dentin *(star)* and lateral branching is found in between the dentinal tubules *(white arrow).* B, Group II (after 4 weeks): shows Dentinal tubules orifices show irregular outline *(white arrow).* Dentin shows widening of dentinal tubules to the extent of becoming connected with the neighboring lateral branches *(head arrows).* C and D, Group III (after 3& 4 weeks): show Irregular deposits appear on the dentin surface that blocked most of the dentinal tubules *(white block arrows).* Regular outline of dentinal tubules, marked decrease in the diameter of dentinal tubules.

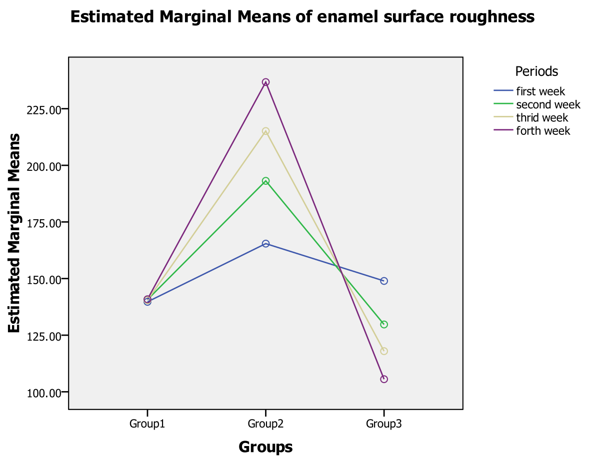

Figure 3: Line chart histogram shows estimated marginal means of enamel surface roughness levels among group I, II and III at different time periods.

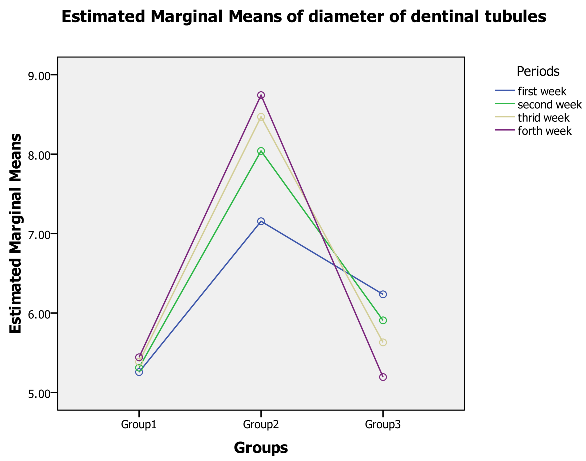

Figure.4: Line chart histogram shows estimated marginal means of the diameter of dentinal tubules levels among group I, II and III at different time periods.

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