

Evaluation of the Effect of Casein Phosphopeptide-Amorphous Calcium Phosphate Mousse versus Natural Raw Fresh Milk on Enamel Surface Roughness After a pH Challenge

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الخلاصة

الأهداف: تهدف هذه الدراسة إلى تقييم تأثير معجون كازين فوسفوبيبتيد - فوسفات الكالسيوم غير المتبلور (CPP-ACP) وحليب الجاموس الطازج الخام وحليب البقر على خشونة السطح لأفات التسوس الأولية الاصطناعية. **المواد وطرائق العمل:** تم جمع 100 ضاحك أولي علوي سليم قلعت لغرض التقييم وتم تقسيمها عشوائياً إلى أربع مجموعات، خضعت الأسنان في جميع المجموعات لإجراء دورة الأس الهيدروجيني ثم تمت معالجتها باستخدام: المجموعة 1: (عينة 25) الماء منزوع الأيونات، المجموعة 2: (عينة 25) معجون كازين فوسفوبيبتيد - فوسفات الكالسيوم غير المتبلور، المجموعة 3: (عينة 25) حليب البقر الخام، المجموعة 4: (عينة 25) حليب الجاموس الخام. تم تقييم سطح المينا بواسطة جهاز اختبار الخشونة عند خط الاساس وبعد نزع المعادن وبعد إعادة التمدن. **النتائج:** في جميع المجموعات كانت هناك زيادة ذات دلالة إحصائية عالية في خشونة السطح بعد إزالة المعادن. وكان هناك انخفاض كبير إحصائياً في خشونة السطح في جميع المجموعات باستثناء المياه منزوعة الأيونات بعد إعادة التمدن، وتم العثور على أعلى تأثير إعادة التمدن في مجموعة معجون كازين فوسفوبيبتيد - فوسفات الكالسيوم غير المتبلور متبوعة بحليب الجاموس الطازج الخام ثم حليب البقر الطازج الخام. **الاستنتاجات:** ضمن حدود هذه الدراسة استنتج أن معجون كازين فوسفوبيبتيد - فوسفات الكالسيوم غير المتبلور وحليب الجاموس الطازج الخام وحليب البقر كانت عوامل فعالة في إعادة التمدن والتي انعكست من خلال تقليل خشونة السطح أفات التسوس الأولية الاصطناعية، ولكن مع إمكانات مختلفة، كان معجون كازين فوسفوبيبتيد - فوسفات الكالسيوم غير المتبلور الأفضل يليه حليب الجاموس الطازج الخام الذي اظهر نتائج افضل بتقليل خشونة سطح المينا مقارنة بحليب البقر الطازج الخام.

ABSTRACT

Aims: The aim of this study is to evaluate the effect of CPP-ACP, raw, fresh buffalo milk and cow milk on surface roughness of artificial initial caries lesions. **Materials and Methods:** 100 sound maxillary first premolar extracted for the purpose of orthodontic treatment had been collected and randomly divided into four groups, in all groups the teeth subjected to pH cycle procedure then treated with: Group1: n(25) deionized water, group2: n(25) CPP-ACP tooth mousse, group3: n(25) raw cow milk, group4: n(25) raw buffalo milk. Enamel surface was assessed by a profilometer device at a baseline and after demineralization and after remineralization. **Results:** In all groups, there was a high statistically significant increase in surface roughness after demineralization. And there was a high statistically significant decrease in surface roughness in all groups except deionized water after remineralization, the highest remineralization effect was found in CPP-ACP tooth mousse group followed by buffalo milk and then cow milk. **Conclusions:** Within the limits of the current study, it was concluded that CPP-ACP tooth mousse, raw fresh buffalo milk and cow milk were effective remineralizing agents which was reflected by decreasing surface roughness of artificial initial caries lesion, but with different potentials, CPP-ACP tooth mousse was the best followed by fresh raw buffalo milk which showed superior results in reducing surface roughness in comparison with fresh raw cow milk.

Key words: Casein phosphopeptide-amorphous calcium phosphate, raw milk, surface roughness, remineralization, caries.

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INTRODUCTION

Dental caries is a main oral health disease which is initiated by demineralization of hard tissue structure of the tooth through organic acids produced from carbohydrates fermentation by cariogenic bacteria in the dental plaque. The presence of sufficient ions of calcium and phosphate in the environment enables the reconstructing of partly dissolved apatite crystals in a process called remineralization ⁽¹⁾. One of the objective of modern dentistry is the noninvasive management of non-cavitated caries lesions through remineralization to prevent disease progression ⁽²⁾.

Casein phosphopeptide amorphous calcium phosphate is a milk based agent in which CPP forms nanoclusters with ACP, CPP–ACP can stabilize calcium and phosphate ions in the solution. Thus providing a pool of calcium and phosphate ions which can maintain them in supersaturation level, also it has buffering action to plaque pH ^(3,4).

In general milk is considered as a protecting agent against dental caries, due to its high level of calcium and phosphorous, and moreover it has protein buffering capacity, especially if

consumed immediately after acid intake ^(5,6). Also, it has been proposed that the fat of milk plays an important role in prevention of dental caries, either by its physical effect by decreasing the adhesion between the food and tooth surface, or by microbiological effect by having a bacteriostatic property ⁽⁷⁾.

Roughness is a fundamental property of teeth, which affects the attachment of exogenous materials to enamel surfaces and caries progression ^(2,8).

The aim of the current study is to evaluate the effect of casein phosphopeptide-amorphous calcium phosphate versus natural raw fresh buffalo and cow milk on enamel surface roughness after a pH challenge.

MATERIALS AND METHODS

Sample Collection:

The sample of this study consisted of (100) human permanent first premolars extracted for the purpose of orthodontic treatment. After extraction, the teeth were cleaned with tap water and examined with 10X magnifying lens, the selection of the teeth followed specific criteria; the teeth must be sound, free from enamel defects, decay, stain,

cracks, demineralization, fluorosis and restorations. The teeth were stored in 0.1% thymol solution.

Sample Preparation:

The teeth were cleaned with rubber cup and non-fluoridated pumice by using low speed hand piece, then the crowns of the teeth were separated from the roots at cemento-enamel junction by using diamond disc in hand piece under copious of water, then the teeth were adapted in a cold cure acrylic mold with specific dimensions (20mm diameter, 15mm depth) in a technique that the buccal surfaces of crowns were exposed and paralleled to the floor. The buccal surface of each tooth was grounded and polished using grit paper (grit 400, 600) ten times in one direction, in order to get a flat surface of each specimen of tooth for surface roughness testing⁽⁹⁾.

Materials:

CPP-ACP Tooth Mousse:

Topical cream with bioavailable calcium and phosphate (GC America, Reclident, Alsip, USA), which contains 10% by weight of CPP-ACP in addition to glycerol, D-sorbitol, CMC-Na, silicon dioxide, propylene glycol, titanium dioxide, xylitol, phosphoric acid, flavoring, zinc oxide, magnesium oxide, butyl p-hydroxybenzoate, guar gum, sodium saccharin, ethyl p-hydroxybenzoate, propyl p-hydroxybenzoate, Pure water.

Milk:

Fresh raw buffalo and cow milk were obtained from local farm dealer (local breed) in Mosul City by single milking dose, the milk was kept cooled in the refrigerator.

Groups Design and Methods:

Group 1: (control negative) n=25 the teeth were subjected to pH cycling procedure for ten days and then were stored in deionized water until subjected to surface roughness test.

Group 2: n=25 the teeth were subjected to pH cycling procedure for ten days and then CPP-ACP tooth mousse was applied. The protocol of application GC tooth mousse in this study was followed the manufacturer's instructions by application thin layer of the cream on each tooth specimen by fine brush for 3 minutes, then again the cream was distributed by the brush and left for 30 minutes. This procedure was repeated two times daily for 7 days⁽¹⁰⁾. Between sessions the teeth were stored in deionized water.

Group 3: n=25 the teeth were subjected to pH cycling procedure for ten days and then, the teeth were immersed in 250 ml of raw fresh cow milk for 50 hours continuously, the milk was replaced every 2 hours⁽¹¹⁾. Then the teeth were stored in deionized water until subjected to surface roughness test.

Group 4: n=25 the teeth were subjected to pH cycling procedure for ten days and then, the

teeth were immersed in 250 ml of raw fresh buffalo milk for 50 hours continuously, the milk was replaced every 2 hours ⁽¹¹⁾. Then the

teeth were stored in deionized water until subjected to surface roughness test. All the groups can be seen in Figure (1).

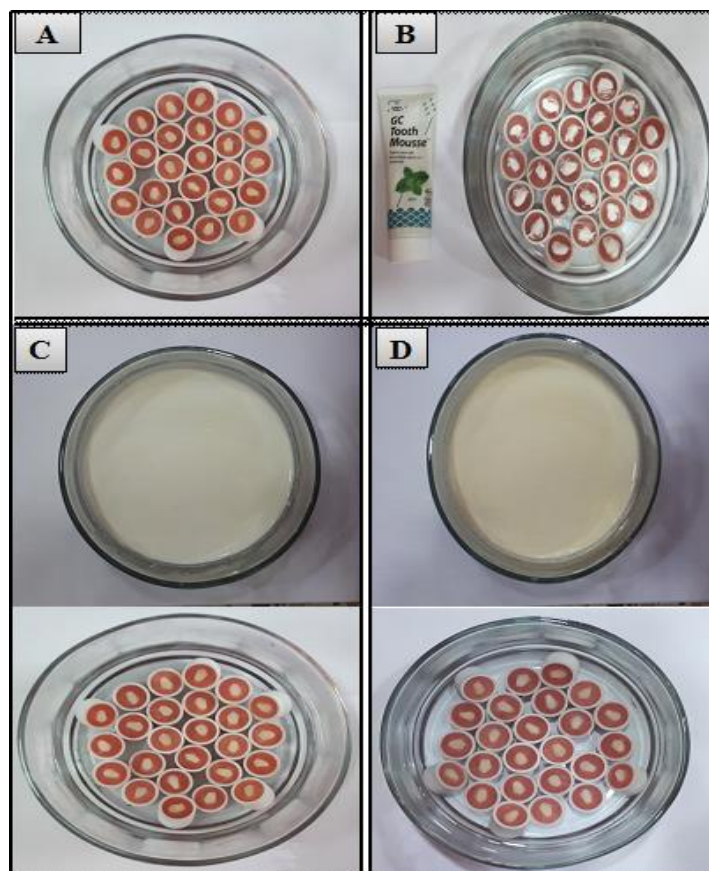


Figure (1): Groups design, (A)Teeth immersed in deionized water after pH cycling, (B) CPP-ACP tooth mousse applied after pH cycling, (C) Teeth immersed in cow milk after pH cycling, (D) Teeth immersed in buffalo milk after pH cycling

Formation of Initial Like Caries Lesion in Enamel Surface of Specimens :

The formation of initial like caries lesion followed the protocol of demineralizing and remineralizing solutions preparation, and adjustment of the pH ^(12,13).The demineralizing solution composed of calcium chloride 1 mM/L, acetic acid 0.075 M/L,

potassium phosphate 2 mM/L and deionized water 1L, while the remineralizing solution composed of potassium phosphate 0.9 mM/L, potassium chloride 150mM/L, calcium nitrate 1.5 mM/L and deionized water 1L, and the pH values of demineralization solution adjusted at 4.3 and the remineralization

solutions at 7 and were measured every day by using pH meter and adjusted if necessary.

The pH Cycling Procedure:

Each group was immersed in 250 ml of the demineralization solution and retained for 6 hours. Then the teeth were removed and rinsed with running deionized water for one minute. After that each group was immersed in 250 ml of remineralizing solution for 17 hours. This procedure was done one time each day and repeated for a period of 10 days^(12,13).

Surface Roughness Test:

For evaluation of enamel surface texture, surface roughness (SR) test was

conducted at Mosul University/Technical Institute/Metallurgy laboratory using a profilometer (Mitutoyo, Tokyo, Japan) with magnification 50X. The surface roughness of the specimens was calculated using the parameter of maximum roughness height R_t which was determined by measuring highest peak (R_p) and lowest valley (R_v) in y direction along the central line of the area using the formula ($R_t=R_p+R_v$) as seen in Figure (2) and the resulted value was expressed in μm ^(14,15).

The cutoff value (reference length) was adjusted at 0.8 mm. Three values of surface roughness for each specimen were taken and the average of these values was considered.

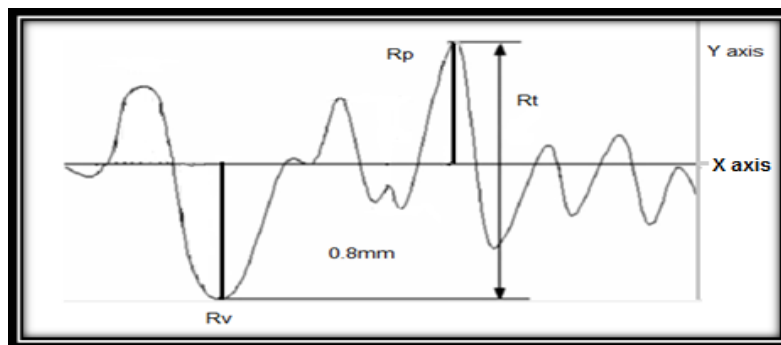


Figure (2): Maximum roughness height (R_t)

RESULTS

Table (1) demonstrates the comparative mean values of the surface roughness (SR) between the groups at the baseline, demineralization and remineralization protocols by one way analysis of variance (ANOVA) test. The results showed that there were no

statistically significant differences in baseline and demineralization values for surface roughness within and between groups $p \geq 0.05$, while there was a highly statistically significant difference in remineralization treatments within and between groups $p \leq 0.01$.

Table (1): ANOVA Test for mean values of SR within and between the groups.

Surface Roughness		Sum of Squares	df	Mean Square	F	Sig.
baseline	Between Groups	0.029	3	0.010	0.359	0.783
	Within Groups	2.614	96	0.027		
	Total	2.643	99			
Demineralization	Between Groups	0.053	3	0.018	0.540	0.656
	Within Groups	3.148	96	0.033		
	Total	3.201	99			
Remineralization	Between Groups	41.179	3	13.726	576.542	0.000*
	Within Groups	2.286	96	0.024		
	Total	43.465	99			

Df: degree of freedom

*Highly significant difference existed, significant difference at $p \leq 0.01$.

Table (2) shows descriptive statistic including mean and standard deviation, Duncan's multiple range test of mean values of SR of the teeth between the groups of different remineralizing treatments. The results showed that there was no statistical significant difference in baseline values with mean surface

roughness ranging between (0.520-0.560), results also showed that in demineralization there was an increase in mean surface roughness with no significant difference in mean roughness scores between the groups and the range between the mean roughness values was between (2.808-2.856).

Table (2): Means values, Standard deviation and Duncan's Multiple Range test of SR mean values of the variables between the groups

Groups	Variables	Baseline	Demineralization	Remineralization
Deionized Water	Mean	0.545 a	2.808 a	2.824 a
	N	25	25	25
	Std.Deviation	0.17723	0.15253	0.10116
CPP-ACP	Mean	0.560 a	2.812 a	1.096 d
	N	25	25	25
	Std. Deviation	0.17078	0.16411	0.20306
Cow Milk	Mean	0.520 a	2.856 a	1.772 b
	N	25	25	25
	Std. Deviation	0.16833	0.22561	0.13699
Buffalo Milk	Mean	0.520 a	2.856 a	1.480 c
	N	25	25	25
	Std. Deviation	0.14142	0.17340	0.15811
Total	Mean	0.536	2.833	1.793
	N	100	100	100
	Std. Deviation	0.16340	0.17982	0.66260

N: Number of the specimens, Std. Deviation: Standard Deviation.

Different small letters indicate statistically significant difference within the same column (Vertically) $p \leq 0.05$.

In remineralization there was a statistically significant difference between the groups, the lowest SR mean value was found in CPP-ACP followed by fresh raw buffalo milk followed by fresh raw cow milk group, while the highest value was found in the deionized water group. However none of the mentioned groups decreased the SR mean values to the baseline.

DISCUSSION

Enamel surface roughness assessment is a useful means of evaluating the stage and activeness of carious lesion. Increase enamel

roughness create favorable conditions to bacterial retention and colonization which is one of the main important etiological factors of caries development, so smoothing out the surface of enamel, can play a major role in disease prevention and ensures a shine and visual acceptance tooth color⁽¹⁶⁾.

Our study demonstrated that demineralization of the enamel surfaces resulted in increased surface roughness expressed in higher roughness values compared with the base line, many studies concluded that increased surface roughness

leads to an increase in susceptibility to bacterial adhesion^(17,18) which in turn will result in an increase in plaque accumulation and biofilm formation and bacterial adhesion which is better formed and accumulated on rougher surfaces which in turn will result in progression of the carious lesion, because these areas are more difficult to be cleaned by the action of saliva or by tooth brushing. Thus, a smoother surface as expressed with a less surface roughness score or with few irregularities as expressed during the remineralization phase of this experiment when the teeth were treated by application of CPP-ACP, buffalo milk and cow milk respectively occurred and is mandatory in the healing process of the initial lesion.

Table (1) showed there was no significant differences $p \geq 0.05$ at baseline and demineralization between the groups due to the standardization and the use of same protocol of pH cycle for all groups, while in remineralization there was highly significant differences $p \leq 0.01$ between the groups due to the differences in the remineralizing ability of each treatment regime.

Table (2) exhibited no significant differences of SR mean values at baseline between the groups. Beside that there was no significant differences between the groups at demineralization due to the use of same pH cycling procedure for all groups.

The remineralization effect of CPP-ACP could be due to the nature of CPP-ACP which is considered as an electroneutral amorphous nanocomplexes. This nanomeasurement property enables calcium and phosphate ions to be released from the remineralizing agent to infiltrate and diffuse surface and subsurface enamel porosities. These ions have a high binding affinity property for apatite so when entering the lesion, they would bind to the more favored surface of apatite crystal^(19,20). In addition, CPP-ACP reduce the lesion depth, improve surface roughness and promote enamel remineralization by deposition of mineral into the porous zone surface of the enamel due to its ability to be as reservoir of bioavailable calcium and phosphate ions^(21,22).

The results of CPP-ACP group is in agreement with Piątek-Jakubek *et al.*⁽¹⁶⁾ that concluded that the demineralized enamel can be improved and smoothed after application of GC tooth mousse for long-term. Also it in agreement with Salama *et al.*⁽⁸⁾ that concluded that there was significantly positive difference of demineralized enamel after application of MI paste in comparison to control negative, although the researchers used primary teeth and 3D optical noncontact surface profiler for assessment.

Milk remineralizing action and decrease surface roughness could be due to its ability to increase mineral and organic material deposit on the enamel surface and a protective film

formation. This film is related with casein adsorption on the enamel surface which decreases dissolving the crystals of hydroxyapatite ^(5,23).

The result of milk is in agreement with Abd-elmonsif *et al.* ⁽¹¹⁾ that studied erosion and concluded that, when demineralized enamel was followed by cow milk exposure, a significant increase in calcium and phosphate levels and a reparative effect on eroded enamel was observed, although the researchers used scanning electron micrographs and energy dispersive X-ray analysis for analysis, the current study also used fresh raw buffalo milk which hasn't been evaluated before for its effect on surface roughness in addition to cow milk and it proved to have superior effect in reducing the surface roughness compared to cow milk. The results of milk and CPP-ACP contradict results of Wiegand and Attin, ⁽²⁴⁾ who concluded that, milk and CPP-ACP were not significantly effective in decreasing enamel loss after specimens were extra orally eroded.

Moreover, Table (2) showed there was significant differences in the remineralizing ability of treatment regimes, the highest decrease in mean values of surface roughness was found in CPP-ACP followed by buffalo milk and then cow milk, while the lowest decrease in mean values of surface roughness value was found in deionized water so that the profilometry evaluation of enamel surface roughness revealed that after applying the teeth

specimens in to natural raw buffalo milk resulted in a significantly greater smoothness with results coming in to the second place after the commercial CPP-ACP group, then came the natural raw cow milk and finally the untreated enamel. Although both types of milk significantly reduced SR, buffalo milk exhibited more promising results than cow milk and this could be due to several biochemical differences between buffalo milk and cow milk, the calcium content is higher in buffalo milk than in milk from cow, and it contains more colloidal calcium and phosphorus, beside that it is richer in proteins, mineral and fat ⁽²⁵⁾. In addition to its protective effect we should not forget that milk in all its types is the arch criminal in development of nursing bottle syndrome which is due to bad feeding habits and is always correlated to improper and prolonged bottle use or breast feeding, particularly at bedtime ⁽²⁶⁾. So the use of milk whether it has a beneficial or harmful effect will depend on the way that it is used.

CONCLUSION

Although in vitro studies have greatly improved our understanding of demineralization and remineralization process, it cannot simulate the complex nature of oral environment. The demineralization process by pH cycling procedure increased surface roughness and created initial like caries lesion on enamel surface. CPP-ACP tooth mousse, raw fresh buffalo milk and cow milk showed

promising remineralizing effects which were expressed by decreasing surface roughness of artificial initial caries lesion, but with different abilities, CPP-ACP tooth mousse was the best followed by fresh raw buffalo milk then fresh raw cow milk.

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