Comparison Study on Casein Phosphopeptide-Amorphous Calcium Phosphate Paste and Fluoride Gel on Remineralization of Demineralized Enamel Lesions

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Abstract

Objective: Tooth surface undergoes continuous remineralization and demineralization. The aim of this study was to compare the effect of Caseinphosphopeptide-amorphous calcium phosphate (CPP-ACP) paste and Fluoride gel on the microhardness of demineralized enamel lesions.

Methods: Frothy eight specimens of premolar teeth were chosen and randomly divided to 4 groups of 12. After the initial measurement of micro-hardness the specimens were immersed in demineralizing solution for 4 day and then the measurements were recorded again. Two groups (GL and G3) were treated with CPP-ACP and Fluoride gel respectively according to manufacturer's instruction. Two other groups (G2 and G4) were treated with CPP-ACP and fluoride gel every week for three months. After the treatments all specimens were taken into PH-cycling and the micro-hardness for each one were measured again. For data analysis, the Repeated Measures ANOVA test and the LSD tests were performed. In each group the percentage of micro-hardness recovery was measured.

Results: Repeated Measures ANOVA and LED Test showed that the mean value of hardness was significantly decreased after demineralization in all groups (p=0.01). There was no significant difference in mean hardness value in groups (GL, G3) after treatment (p=0.1, p=0.12) In groups (G2, G4) the mean hardness value were significantly increased (p<0.0001, p=0.1). It is noticeable that the CPP-ACP was significantly more efficient than the fluoride gel.

Conclusion: CPP-APC paste and fluoride gel both increase the micro-hardness of enamel when administrated for long time and repeated application.

Key words: Dental Enamel, Private office, Tooth Demineralization, Tooth Remineralization.

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Introduction:

Dental structure within the oral environment is constantly exposed to demineralization and remineralization, and if the existing equilibrium is disturbed for any reason, it will result in destruction of tooth structure (1). A carious lesion begins with establishment of a combination of specific bacterial populations. In a neutral environment, the enamel hydroxyapatite crystals are in equilibrium with the saliva, which is saturated of calcium and phosphate ions (2). In pH=5.5 and below, hydrogen ions produced by bacterial metabolism, react with phosphate group of enamel crystals. The result of these reactions is

the dissolution of enamel or enamel demineralization. If the pH level comes back to neutral, and calcium and phosphate ions are available in the surrounding environment, the enamel dissolution can be reversed. The primary enamel lesions are capable of remineralization, especially with using booster remineralization therapies (3).

Fluoride is the most common material used for enamel remineralization. This substance has beneficial effects in reducing tooth decay through reducing acid production by microorganisms, inhibition of their intracellular and extracellular enzymes and replacing in the crystal structure of hydroxyapatite and converting it into fluorapatite resistant to acid (4). However, the use of fluoride can cause unwanted side effects like fluorosis and toxicity in high doses (5). Thus, the scientists are still trying to find effective anti-caries compounds without the adverse effects of fluoride.

In this regard, several studies have shown that milk and its derivative products like cheese have anti-caries property in humans and animal models, which mechanism of action is due to chemical effects of casein phosphoprotein and the calcium component in cheese (6). Caseinphosphopeptide-amorphous calcium phosphate (CPP-ACP) complex applies its protective anti-caries effects through inhibition of demineralization or a combination of increased remineralization and reduced demineralization (7-10). Each of the functional potentials of CPP-ACP is similar to the effects of the most common anti-cavity ingredient, i.e. fluoride. In addition, CPP-ACP is safe for the teeth, tastes good and is well tolerated by people. Also, unlike fluoride, ingestion of any amount of the substance is harmless and shows none of the adverse effects of overusing fluoride (11-13). Thus, it seems that if the adequacy of this substance in increasing the remineralization process and reducing the demineralization of dental hard tissue is proven, it could be a good alternative for a variety of fluoride compounds. Since there is a relationship between the surface hardness of enamel and the amount of demineralized mineral content, this factor can be used as a criterion for determining the rate of demineralization and remineralization of enamel (14, 15).The purpose of this study was to compare the effects of using CPP-ACP paste (Japan, GC Tooth Mousse) and fluoride phosphoric acid gel (APF) (TOPICAL APF GEL Sultan, USA) on the remineralization of enamel lesions by measuring the hardness rate of enamel following its demineralization by a caries like process in oral cavity.

Methods:

In this experimental-clinical laboratory-type study, over 48 human healthy permanent premolars extracted for orthodontic treatment in patients, which had no caries, restorations, cracks, wear and enamel hypoplasia among the teeth collected and stored in the solution of thymol0.2% at room temperature during about 4 months were selected. The teeth were wiped out by the scalpel blade from contaminations, and then were cleaned using the hand-piece brush with slow speed and water. Then, the roots were cut with a diamond disk and were stored in distilled water at room temperature.

Then, each of the specimens was mounted in special plastic mount cylinders 5.2 cm in diameter using epoxy glue. For mounting, a plate with a square hole by dimensions of 2×2 mm created in its center was placed at the bottom of the mount cylinder. Then, the crown was placed inside the mount cylinder in such a way that its buccal surface is located in the center of the square. Following the procedure, to enhance the micro-hardness measurement accuracy, the enamel samples were polished by abrasive paper (Silicone carbide, USA, 4000 grit) to create a plane and smooth surface. The surface of each sample was also examined using

a microscope with a magnification of 1000X in terms of smoothness, and if necessary, was re-polished. It should be noted that in case of the exposed dentin surface, the sample was excluded.

All samples were randomly divided into four 12-item groups, and then, the primary surface micro-hardness of the polished center portion of each sample was measured by the Vickers hardness measurement machine (digital microhardness tester, Buhler, Germany) (T1). Then, to produce caries, the samples were incubated at 37 C° for 4 days (96 hours) in the demineralization solution with a formula of Lactic acid (0.05mM), (2.2mM) NaH_2PO_4, ($CaCl_2$ (2.2mM) and Fluoride (0.2ppm) to adjust its pH was to 5.4 with NaOH 50%.

It should be mentioned that the solutions were changed every day to prevent the accumulation of substances resulting from demineralization and changes in pH. After 96 hours, initially the surface of each of the samples was thoroughly rinsed by rinsing syringes containing artificial saliva, Na_3PO_4 (3.9mM), $NaCl_2$ (4.2mM), KCl (17.9mM), $CaCl_2$ (1.1mM), $MgCl_2$ (0.08mM), H_2SO_4 (0.5mM) and $NaHCO_3$ (3.2mM), and then all of them were kept in containers containing artificial saliva, and the surface micro-hardness was measured again (T2).

First, the specimens were air-dried for 30 seconds. Then, in the first group (G1), the CPP-ACP paste was placed for 3 min. In the second group (G2),the specimens were treated with CPP-ACP paste regimen once a week for 3 months for 3 minutes. In the third group (G3), the specimens surfaces were treated by fluoride gel (APF) for 4 min. In the fourth group (G4),the specimens were treated with fluoride gel (APF) regimen once a week for 3 months, each time for 4 minutes.In all groups, after placement of these materials, the teeth were cleaned by cotton rolls, and then immersed in artificial saliva. After one hour, they were removed from saliva, and the remaining material was cleaned using a scalpel blade, No. 15 slowly from the enamel surface. The surface of each sample was rinsed using a syringe containing artificial saliva. To measure the resistance of the treated samples to demineralization and simulation of oral conditions, the specimens of all groups were immersed in artificial saliva solution for 21 hours(as remineralization solution), and then PH Cycling was applied by immersing them for 3 hours in the demineralization solution at 37 °C for 5 days. Then, the following tests were carried out: Microhardness test (T3);initial enamel microhardness to compare the mean (T1); microhardness after initial demineralization (T2); after applying the treatment regimes and PH Cycling (T3), the ANOVA test was used to study the groups. The one-way variance analysis test was used for micro-hardness mean comparison between different groups. To compare the mean micro-hardness between different times in each group, the ANOVA with repeated observations was used. For data analysis, the statistical SPSS software, Ver. 16 was used.

Results:

The mean values of micro-hardness at three different times in different groups are given in Table 1. According to the data of this study, Repeated Measures ANOVA and the LSD post hoc test showed that the mean micro-hardness in G2 group (frequent and prolonged use of CPP-ACP) was significantly higher than all the groups (p=0.03) and, in group G3 (once using fluoride gel) significantly lower than other groups (p=0.34). The mean micro-hardness in group G4 (frequent and prolonged use of fluoride gel) was slightly higher than the group G1 (once using CPP-ACP), whereas there was no significant difference (p=0.6).

Table 1- Mean microhardness of enamel at three times in groups G1, G2, G3, G4

Groups	Before Demineralization (T1)	After demineralization (T2)	After treatment (T3)	p-value
	mean (SD)	mean (SD)	mean (SD)	
G1	337.72 (71.14)	261.04 (67.17)	272.21 (66.56)	0.005
G2	300.18 (58.58)	256.64 (59.34)	357.37 (46.98)	<0.001
G3	330.11 (44.94)	232.64 (49.24)	239.67 (47.89)	0.001
G4	394.42 (86.96)	243.74 (47.13)	285.71 (52.56)	<0.001
p-value	0.31	0.601	<0.001	-

In all groups, the mean micro-hardness at time T2 (G1 (p=0.003), G (p=0.01), G3 (p=0.01), and G4 (p=0.004)) significantly decreased compared to T1 (p=0.01). At T3 compared to T2, no significant difference was seen in groups G1 (p=0.12) and G3 (p=0.1); however, there was a significant increase in micro-hardness in two groups of G2 (p<0.001) and G4 (p<0.001). Comparing the mean micro-hardness of T3 and T1, it was shown that that there was a significant reduction in groups G1 (p=0.01) and G3 (p=0.002).However, the difference was not statistically significant in group G4 (p=0.67), while a significant increase was seen in group G2 (p=0.001). The One Way ANOVA test showed in comparison the microhardness of 4experimental groups at T1 (p= 0.31) and at T2 (p=0.601), there was no significant difference; but at T3, the difference was significant (p<0.001). The G2 group had significantly higher microhardness than the other groups (p=0.04).

Discussion:

Today, various therapeutic agents have been introduced in order to restore the early enamel lesions and its remineralization. Fluoride (in different forms) is used for years as the most common substance to prevent and remineralize the enamel (4). The CPP amorphous calcium phosphate complexes, known for short as CPP-ACP, is considered as other preventive substances that has drawn considerable attention recently attracted (1, 7, 8-10). Considering the relationship between the enamel surface micro-hardness and its mineral content, this factor can be used as a measure to compare these two substances to stop the decay process (demineralization) and restore the lesions (remineralization). The surface micro-hardness is a simple, non-destructive and rapid method for demineralization and remineralization studies (3).

In this study, in two G1 and G3 groups, after creating artificial caries lesions, the treatment regimens of CPP-ACP and fluoride gel were respectively used for 3 and 4 minutes according to the manufacturers' instructions for professional use of these materials in office. Despite a low increased remineralization and microhardness of enamel, no significant difference was seem between them in this regard (CPP-ACP and fluoride gel caused a slight increase in micro- hardness, which was not significant). This may be due to insufficient exposure time of the specimens to the substances. Thus, in case of professional use of these materials, their repeated application at shorter intervals is recommended.

In G2 and G4 groups, after developing caries lesions, the CPP-ACP and fluoride gel treatment regimens were respectively used, each for 3 months, once a week were according to the manufacturer's instructions. The reason for selecting these regimes was using these materials frequently in clinics, especially in children with high caries risk. Thus, applying special trays developed for this by the dentist, the teeth surfaces are stained. The results

showed that both treatment regimens resulted significantly in remineralization of enamel lesions and increased the microhardness of enamel.

Some researchers, in spite of different treatment regimes and different measurement methods, came to almost similar results. Hegde and Moany (2012) in his study concluded that the CPP-ACP significantly remineralizes synthetic enamel subsurface lesions, and the remineralization rate increases with increasing duration of treatment with CPP-ACP (16). Srinivasan (2010) concluded in his study that the CPP-ACP paste alone and also associated with fluoride 900 ppm is capable of softened enamel remineralization, and the potential was greater in the group where CPP-ACP and fluoride were used together (17). It was found in Panich and Poolthong (2009) study that Cola drinks significantly reduce the hardness of the enamel; but, the CPP-ACP and also CPP-ACP with artificial saliva significantly increase the hardness of destructed enamel that the increased hardness was significantly higher than the technique using artificial saliva alone (18). Rahiotis and Vougiouklakis (2007) concluded in his study that the presence of CPP-ACP on dentin surface can reduce demineralization and increase remineralization (10). The results of these studies are consistent with the results of our study.

Kim, *et al.* (2013) demonstrated in their study that the microhardness of specimens treated with 2.2% fluoride polyvinyl alcohol (F-PVA) tape and fluoride varnish was significantly higher than CPP-ACP group that the feature is comparable to the F-PVA fluoride varnish capability of inhibiting demineralization (19). The results of Lata, *et al.* (2010) study using the measurement of micro-hardness show that CPP-ACP is effective in remineralization of early surface lesions, but with less effectiveness than the fluoride, and the combined treatment of CPP-ACP and fluoride creates greater remineralization than the fluoride alone. Also, fluoride, CPP-ACP and a combination of both were ineffective in remineralization of primary enamel lesions depth (3).Pulido et al. (2008) by examining the inhibitory effect of CPP-ACP paste (GC-America) ,sodium fluoride 5000 ppm (colgate)) and sodium fluoride 1100 ppm (crest) and the artificial saliva on caries-like enamel lesions through measuring the depth of the lesions came to the conclusion that there was no significant difference between artificial saliva and CPP-ACP paste, and also between sodium fluoride 1100 ppm and the combined treatment with CPP-ACP, and the CPP-ACP was alone. Finally, high concentrations of sodium fluoride (ppm 5000) showed the greatest reduction in lesion progression (20).

In Kim, *et al*. (2013), Lata, *et al*. (2010) and Pulido, *et al.* study (2008), the effect of fluoride (either in varnish or toothpaste form containing fluoride) on increased remineralization was mentioned equal to or greater than CPP-ACP. This difference may be due to either the short periods of treatment and lack of sufficient time for greater penetration of material to the depth of lesions (especially in the case of CPP-ACP) or using distilled water instead of artificial saliva (3, 19, 20).

In the present study, although both materials significantly increased the microhardness of enamel lesions, but the effect of CPP-ACP in the increase of microhardness was significantly more than the APF gel, increasing the microhardness of enamel comparing to the time before creation of artificial caries. Agnihotri, *et al.* (2012) and Maliki-pour, *et al.* (2012) also reported CPP-ACP paste more effective in increasing the remineralization (21, 22). Kumar, *et al*. (2008) also reported higher reduction in the depth of lesion in CPP-ACP than fluoride (23). Perhaps the reason can be attributed to the ultrafine particles of amorphous calcium phosphate (nano-complexes) in the CPP-ACP paste that their calcium and phosphate ions

easily penetrate into the porous lesion and infiltrate to the depth of demineralized lesion due to very small size (nanoscale size), and reform the apatite crystals even in the deeper parts, which is however subject to long-term treatment with these materials and having sufficient time for exposure. While fluoride, although increasing the resistance (strength) of tooth surface against demineralization through sedimentation of apatite deposits, however, the resulting remineralization despite its benefits is a self-limiting phenomenon, which prevents the infiltration of calcium and phosphate ions into the depth of the lesion (3, 4). The foregoing may explain the higher impact of CPP-ACP on increasing surface micro-hardness than the fluoride gel.

It should be noted Panich and Poolthong (2009) also examining the demineralized enamel hardness showed that in addition to that CPP-ACP increases the microhardness of the demineralized enamel, the impact of this substance is increased substantially with artificial saliva and has a synergistic effect. Thus, the in case of immersion of samples impregnated to the substance in artificial saliva compared to distilled water, the microhardness increase will be greater (18, 22), which is consistent with the manufacturer's a claim implying that the saliva enhances its effect, and as the paste remains more in the mouth, its effects will enhance. It is also consistent with the present study that tries to simulate the oral environment and saliva (In this study, the microhardness number valued to 357.37 that was even higher than the healthy enamel).

In the present study, the samples of all groups were exposed to PH Cycling for 5 days to measure the resistance of samples to demineralization, and then, the hardness measurement test was performed. The results of statistical data showed that treatment with CPP-ACP was significantly more effective than fluoride regimen on increasing the tooth resistance to the demineralization.

In this study, the percentage of surface microhardness recovery was calculated in all groups. This percentage represents the rate of microhardness restoration and the rate of remineralization of demineralized enamel after the used regimen treatment. This percentage is also used to compare the effects of various substances on enamel remineralization.

In the present study, calculating the percentage of surface microhardness recovery showed that frequent and prolonged use of CPP-ACP significantly improves the hardness restoration so that the enamel hardness increased after treatment with this paste compared to the healthy enamel (before demineralization) (231.23%). Being higher than 100 regarding CPP-ACP indicates that the resulted hardness after treatment regimens is even greater than the initial state (initial enamel hardness). In case of APF, the recovery percentage shows that although this material led to the return of microhardness to the rate of 82.81%,but the value is significantly less compared with the CPP-ACP.

Conclusion:

CPP-APC paste and fluoride gel both increase the micro-hardness of enamel when administrated for long time and repeated application.

Conflict of Interest: “None Declared”

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