Original Article

Effect of Fluoride, Casein Phosphopeptide–Amorphous Calcium Phosphate and Casein Phosphopeptide–Amorphous Calcium Phosphate Fluoride on Enamel Surface Microhardness After Microabrasion: An In Vitro Study

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Abstract

Objectives: This study aimed to assess the effect of applying casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) paste, casein phosphopeptide–amorphous calcium phosphate fluoride (CPP-ACPF) paste and sodium fluoride gel on surface microhardness of enamel after microabrasion.

Materials and Methods: Thirty freshly extracted human premolars were selected. All samples were subjected to hardness indentations made with the Vickers hardness machine and the average value was recorded as the initial surface microhardness. The specimens were then randomly divided into three groups (n=10) of CPP-ACPF, fluoride and CPP-ACP. The teeth were micro-abraded with Opalustre. Microhardness test was performed to assess the post-abrasion hardness. Three remineralization modalities were performed on samples of each group. The enamel surface microhardness measurements were performed. To compare the difference between groups, the rehardening and softening values were defined. One-way ANOVA and Tukey's post hoc test at a significance level of 5% were used for statistical analysis.

Results: The mean microhardness value (MMV) had a significant decrease after microabrasion from baseline. The MMV had a significant increase after remineralization in all groups. The MMV of CPP-ACPF group was significantly more than that of fluoride group (P=0.027). The rehardening value of fluoride group was significantly more than that of other groups (P<0.001).

Conclusion: All the remineralizing agents were effective for rehardening the enamel after microabrasion. The CPP-ACP and CPP-ACPF pastes are effective, but to a lesser extent than neutral sodium fluoride gel in remineralizing enamel surface. Incorporation of fluoride to CPP-ACP formulation does not provide any additional remineralizing potential.

Keywords: Casein phosphopeptide-amorphous calcium phosphate nanocomplex; Enamel Microabrasion; Hardness; Sodium Fluoride

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INTRODUCTION

Enamel microabrasion is a conservative approach in order to improve surface properties, remove superficial discolorations and eliminate enamel decalcification [1]. This procedure is used only for stains or defects that are no deeper than few tenths of a millimeter in enamel [2]. McCloskey [3] first introduced this

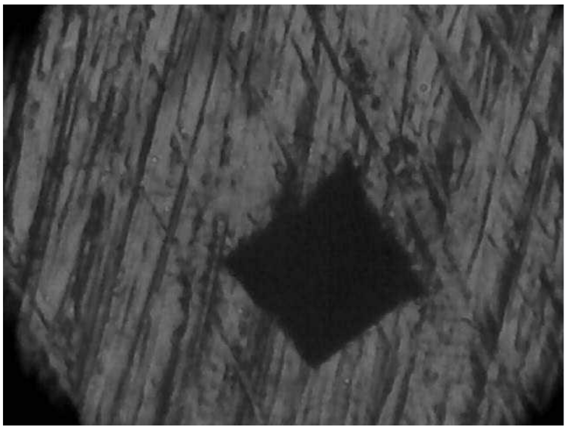

Fig. 1. Indentation made with the Vickers hardness machine

technique using 18% hydrochloric acid to remove superficial tooth structure in teeth affected by fluorosis.

Later, Croll and Cavanaugh [4] recommended a technique in which a paste composed of hydrochloric acid (HCL) in combination with pumice was applied to the affected area with a tongue blade. This technique was further modified by Croll [5], replacing the pumice paste with silicon carbide particles and reducing the concentration of HCL to 11%. The combined effects of acidic solution and abrasive particles erode the enamel surface [6]. Today, commercially available products provide improved and safer means for microabrasion[2].

Microabrasion eliminates the outer layers of enamel leaving a smooth, glossy enamel surface; although microscopic roughness may be seen on the enamel due to acidic nature of the blend [1,7]. Ulukapi reported a significant reduction in enamel microhardness following microabrasion, which continued for two days and could increase the risk of enamel erosion during this period [7]. In order to enhance remineralization after microabrasion, it is recommended to polish treated areas with fluoride prophylactic pastes and to apply topical fluoride on the tooth surface [7,8]. Even though fluoride can promote remineralization, the rate depends on pH of the solutions, fluoride concentration, type of fluoride salt and presence of mineral ions in the saliva [9]. Applying a neutral sodium fluoride gel on enamel for four minutes after microabrasion has been suggested in a previous study [7].The CPP–ACP nanocomplex derived from milk protein was introduced as a supplemental source of calcium phosphate ions [10,11]. These nanocomplexes act as calcium and phosphate reservoirs and keep supersaturated levels of these ions in the oral

Table 1. Composition and manufacturers of the materials used in this study

Material	Manufacturer	Composition
Tooth Mousse	GC Corporation, Tokyo, Japan	Casein phosphopeptide-amorphous calcium phosphate
MI Paste Plus	GC Corporation, Tokyo, Japan	Casein phosphopeptide-amorphous calcium phosphate with sodium fluoride 900 ppm
MASTER-DENT	Dentonics Inc., Monroe, NC, USA	2% neutral sodium fluoride gel 9000 ppm
Opalustre	Ultradent products, South Jordan, UT, USA	6.6% hydrochloric acid and microparticles of silicon carbide water soluble paste (20–160 μm)
Diamond Excel	FGM, Joinville, SC, Brazil	Micronized diamond, lubricant base, thickener and emulsifier

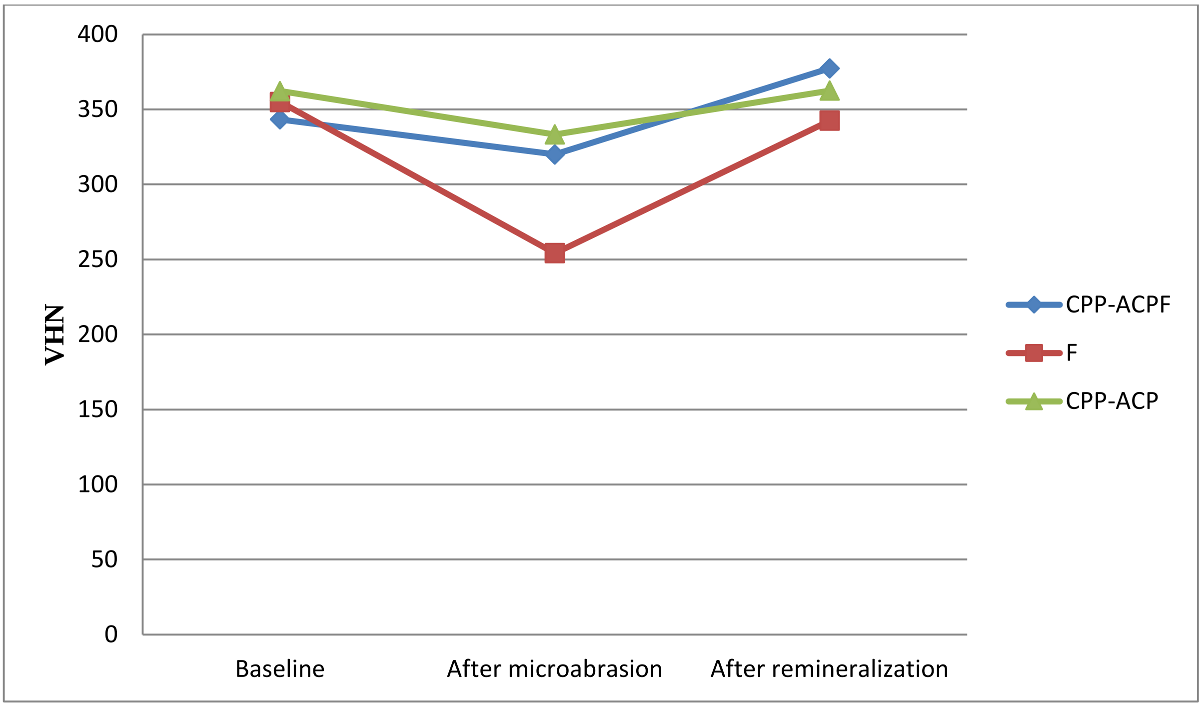

Fig. 2. Change in the enamel surface microhardness (VHN) at the three time points.

cavity when applied on the tooth surface [11]. Also, CPP–ACP has been shown to enhance remineralization and increase the microhardness of eroded enamel [10,11]. Mathias et al. reported a reduction in surface roughness of micro-abraded enamel after a combination of microabrasion and CPP-ACP application [1].

A new product (MI Paste Plus) containing both CPP-ACP and fluoride (CPP-ACPF) was recently introduced. The level of fluoride in this paste is 900 ppm, which is fairly close to that of adult toothpastes. The combination of CPP-ACP and fluoride probably results in co-localization of calcium and phosphate ions with fluoride ions on the enamel surface and reforms the apatite crystals [12-14]. Surface microhardness measurement is a suitable technique that allows uncomplicated and nondestructive evaluation of demineralization and remineralization [15]. Therefore, the purpose of this study was to assess the effect of applying CPP-ACP paste, CPP-ACPF paste and sodium fluoride gel on surface microhardness of enamel after microabrasion.

MATERIALS AND METHODS

In this study, 30 freshly extracted human premolars, without cracks or erosion, were selected and kept in 0.1% thymol solution for one week. The teeth were then cleaned with water and embedded horizontally in self-cure acrylic resin so that the labial surfaces were exposed and parallel to the applied force during the microhardness test. Surface of the samples was ground successively with polishing disks (Soflex Polishing Disks, 3M ESPE, St. Paul, MN, USA) and diamond polishing paste (Diamond Excel, Diamond Excel, FGM, Joinville, SC, Brazil) with a particle size of 2-4 µ in order to obtain a smooth flat area of 3mm × 3mm. The area was outlined using a knife-edge diamond bur. All samples were subjected to hardness indentations made with the Vickers hardness machine (MHZ, Koopacompany, Mashhad, Iran) using a 500 g load and a dwell time of 10 seconds (Fig.1). Three indentations were made for each sample and the average value was recorded as the initial surface microhardness. The specimens were then randomly divided into three groups, with 10 samples per group. After drying the samples by mild flow of air, microabrasion paste (Opalustre, Ultradent Products Inc., South Jordan, USA) containing 6.6% hydrochloric acid and silicon carbide nanoparticles (Table 1) was applied on all samples at one millimeter thickness. The procedure was done by OpalCups (Ultradent Products Inc., South Jordan, UT, USA) latch-type bristle polishing cups and handpiece at a low speed for 60 seconds. After microabrasion, the paste was removed from the surface and the samples were cleaned under running water. Microhardness test was performed with the same technique mentioned before to assess the post-abrasion hardness. Three remineralization modalities were performed on samples of each group.

Group 1: The specimens were treated with 2% neutral pH sodium fluoride gel (MASTER-DENT, Dentonics, Inc., Monroe, NC, USA) for four minutes and were then rinsed.

Group 2: All the samples in this group were treated with MI Paste (GC Corporation, Tokyo, Japan) containing CPP-ACP. The paste was applied directly on the labial surface of the tooth for four minutes, and then washed.

Group 3: In this group, after microabrasion, MI Pated Plus (GC Corporation, Tokyo, Japan) containing CPP-ACPF was used similar to the second group. To standardize the amount of remineralizing agents applied to all samples, 0.3 mL of each was measured by a measuring spoon.

After the remineralization process was completed, the specimens were washed with water and blot dried. All 30 specimens were subjected to microhardness test as mentioned earlier. In order to compare demineralization and remineralization between groups, the formulas mentioned below were used to measure the softening and rehardening values [7].

Softening value = B/A× 100

Rehardening value = C/B× 100

A: Initial microhardness

B: Microhardness after microabrasion

C: Microhardness after remineralization

Statistical analysis was performed with SPSS software (version 22). One-way repeated measures ANOVA was used to compare the surface microhardness and softening and rehardening values at baseline, after microabrasion and after remineralization with significance set at $P<0.05$. The post-hoc Tukey's test was used to find significant differences between the means when ANOVA result was significant.

RESULTS

The MMV at baseline, after microabrasion and after remineralization are shown in Table 2 and Fig.2. The baseline MMV (± standard deviation; SD) was 353.5±35 Vickers Hardness Number (VHN).

The baseline MMVs were not significantly different among the study groups. After microabrasion with Opalustre, the MMV

Table 2. The mean± standard deviation (SD) of surface microhardness value at baseline, after microabrasion and after remineralization (N=30)

Group	Condition (Mean VHN±SD)		
	Baseline	**After microabrasion**	**After remineralization**
CPP-ACPF	343.30±24.130^{aA}	320.00±19.090^{aB}	377.30±23.519^{aC}
Fluoride	355.00±38.618^{aA}	254.20±27.349^{bB}	342.50±29.684^{bA}
CPP-ACP	362.20±43.202^{aA}	333.30±49.137^{aB}	362.50±30.913^{abA}

*Different upper-case letters represent statistical significance within the row, different lower-case letters show statistical difference in the column.

(±SD) was 302.5 ±48.2 VHN that shows a remarkable decrease from baseline. This decrease was significant in all study groups. Softening and rehardening values for each study group are presented in Table 3. The softening of fluoride group was significantly more than CPP-ACP and CPP-ACPF groups (P<0.001).

After remineralization, the MMV increased significantly compared to the values after microabrasion in all groups. The MMV of the group treated with CPP-ACPF was significantly more than that of fluoride group (P=0.027). However, the difference between CPP-ACPF and CPP-ACP groups and the difference between fluoride group and CPP-ACP group was not significant. The rehardening value of fluoride group was significantly more than that of other groups (P<0.001).

DISCUSSION

In this study, the effect of microabrasion on enamel microhardness and the effect of different remineralizing agents on micro-abraded enamel were assessed.

In the current study, the mean VHN of samples at baseline was 353.5 ±35, which meets the VHN range of normal enamel [15]. According to the results obtained, enamel microabrasion with 6.6% HCL and nanoparticles of silicon carbide caused a reduction in microhardness immediately after treatment in all study groups. This finding is in accordance with that of other studies [6,7,16]. Meireles et al. reported that microabrasion with HCL significantly removed the superficial enamel layer and caused mineral loss from the enamel [6].

Different amounts of enamel loss have been reported in previous studies subsequent to microabrasion [16-19].

Several parameters such as the amount of acid used, its concentration and pH, abrasive medium, time of instrumentation and the application force can affect the amount of enamel erosion during microabrasion treatment [16]. In the current study, softening value in the fluoride group was significantly higher than in the other two groups. This may be due to the difference in the amount of force applied. In our study, the groups were matched in terms of the acid amount and the time of instrumentation; however the force applied was uncontrollable. It has been shown that increased pressure results in higher substance loss. Utilizing a load cell could have distributed the load equally between the samples [17].

According to the outcomes, all the remineralizing agents used in this study were able to re-harden the enamel surface after microabrasion. Our findings are consistent with the findings of many studies demonstrating remineralization effect of fluoride, CPP-ACP and CPP-ACPF after erosive procedures [9,10,12-14]. The mean rehardening value in the group treated with neutral sodium fluoride was noticeably higher than that in the other groups, which means that the neutral sodium fluoride was the most effective remineralizing agent. Many studies showed convincing evidence for the preventive effect of fluoride by promoting remineralization [13,14,20].

One of the protective mechanisms of fluoride is formation of a calcium fluoride (CaF2)-like layer on the tooth surface enhancing reconstruction of minerals such as fluorapatite or fluorohydroxyapatite on the surface of enamel crystals [21].

Lata et al. studied the remineralization potential of fluoride and CPP-ACP for enamel lesions. Similar to the current study, they found that fluoride was more effective in remineralization

Table 3. Softening and rehardenig values (%) in the study groups

Group	Softening value	Rehardening value
CPP-ACPF	93.5687	118.3341
Fluoride	71.9998	135.5053
CPP-ACP	91.8457	110.0639

although CPP-ACP had the ability to re-harden the enamel [15]. Rirattanapong et al. found no difference in the remineralizing potential of fluoride toothpaste, CPP-ACP and CPP-ACPF dental products. This may be due to higher concentration of fluoride in our study (9000 ppm) compared to fluoride toothpaste (1000 ppm) in their study [14].

The CPP-ACP paste caused an increase in enamel microhardness after microabrasion with Opalustre. The remineralization effect of CPP-ACP paste involves the incorporation of CPP part of this complex to the enamel and biofilm, in order to deliver the calcium and phosphate ions to the enamel rods and reconstruct the apatite crystals [12]. Our findings are similar to those of Tantbirojn et al, who showed that softened enamel was significantly hardened after being treated with CPP-ACP paste [10].

In CPP-ACPF treatment group, the mean rehardening value was higher than that in CPP-ACP group. However, this difference was not statistically significant. It is speculated that the difference in the percentage of microhardness recovery is caused by the fluoride ions used in CPP-ACPF paste.

Our findings are consistent with those of Rirattanpong et al who reported no additional effect for use of CPP-ACP plus fluoride ions in comparison with using CPP-ACP alone [13,14].

Contrary to our results, the synergistic effect of CPP-ACP and fluoride and greater remineralizing potential of this compound for eroded enamel were confirmed in the study by Srinivasan et al [22]. This contradictory result may be due to different study designs. Future clinical studies are recommended since remineralization in vitro may be quite different from what usually occurs in vivo.

CONCLUSION

Within the limits of the present study, it can be concluded that all the remineralizing agents used were effective in rehardening enamel after microabrasion. The CPP-ACP and CPP-ACPF pastes are effective, but to a lesser extent than neutral sodium fluoride gel for remineralizing the enamel surface. Incorporation of fluoride in the CPP-ACPF formulation does not provide any additional remineralizing potential when compared to CPP-ACP paste.

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