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Microhardness of Artificially Demineralized Enamel treated with Different Regimes of ACP-CPP and Fluoride Agents

Microhardness of Demineralized Enamel using ACP-CPP and Fluoride

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ABSTRACT

Aims: The purpose of this study was to evaluate the microhardness (MH) of artificially demineralized enamel treated with different regimes (CPP-ACP paste, sodium fluoride solution (NaF), and a mixture of CPP-ACP and NaF).

Materials and Methods: Fifty sound human premolars were used in this study. The root discarded and crowns sectioned in mesiodistal direction to yield two pieces. Buccal halves were used in the study. Specimens were mounted inside acrylic and polished to obtain flat enamel surface, specimens were divided into 5 groups (10 teeth in each) as follow: (A) control +ve, (B) control –ve, (C) CPP-ACP, (D) NaF, and (E) CPP-ACP mixed with NaF. Specimens in all groups except A were demineralized with buffered demineralized solution for 2 weeks. Following demineralization, enamel surfaces were remineralized for 5 minutes once a day for 2 weeks according to their group. All groups were then subject to PH cycle for 2 weeks. The MH of the specimens was evaluated using Vicker microhardness testing machine. The data analyzed statistically using one way ANOVA and Tukey test ($p<0.05$).

Results: CPP-ACP mixed with NaF and control +ve exhibited significant MH than CPP-ACP, NaF, and control –ve. But significantly there is no difference between CPP-ACP mixed with NaF and control +ve. Also results show that NaF and CPP-ACP had MH significantly higher than control –ve, while there was no significant difference between them.

Conclusions: The findings of the current study proved the combined benefits of CPP-ACP and NaF in enhancing the microhardness of the demineralized enamel more than if the CPP-ACP or NaF was used alone.

Key words: Microhardness, NaF, CPP-ACP, Demieneralized enamel.

INTRODUCTION

Scientific advances in restorative materials and technique, as well as in understanding pathogenesis of caries, have led to more efficient oral health management.(1) Caries is a chronic, slowly progressing disease, with symptoms not detected at the onset of the disease but generally much later. Its initiation is associated with demineralization of calcium and phosphate, resulting in the formation of a subsurface lesion. It is, therefore, very important to detect caries in its early stage, when the lesion can be reversed clinically by using, for instance, different supplemented procedures and materials to replace the lost minerals.(2)

Several studies have reported the effectiveness of topical fluoride as a cariostatic agent in enhancing enamel remineralization of early caries lesions and reducing tooth enamel solubility. (3,4,5,6,7)

Possible drawback of fluoride remineralization which led to search for other material. In recent years, tooth Mousse has been developed by Prof Reynolds at the University of Melbourne in 1998, which is commercially available casein phosphopeptide (CPP), stabilized amorphous calcium phosphate (ACP) product. CPP-ACP is a useful cariostatic agent for the control of dental caries, CPP stabilizes ACP and forms Nano complexes with ACP at the tooth surface thereby providing a reservoir of calcium and phosphate ions which favours mineralization. CPP also buffers the pH of plaque, depresses demineralization and enhances remineralization which also results in the anticariogenic property of CPP-ACP, and it can be used as an adjunct preventive therapy to reduce caries in high risk patients, to reduce dental erosion in patients with gastric reflux or disorders, to reduce decalcification in orthodontic patients, to repair enamel in cases involving white spot lesions, orthodontic decalcification or before and after tooth whitening and to desensitize teeth. [8,9,10,11]

Little information available in the literatures about the combined effect of CPP-ACP and fluoride as two separate products in enhancing remineralization and anticariogenic effect. [12,13]

Demineralization of the enamel surface in the early stage of caries lesion would reduce surface hardness of the enamel. Among laboratory studies, MH measurement is a simple and reliable method to determine the mechanical properties of enamel following changes in mineral content. [14,15,16,17]

The null hypothesis were CPP-ACP would have no effect on remeralization of enamel and subsequently on the MH, and the combined effect of CPP-ACP and NaF would have no better remineralization effect and MH than independent application of any of the two agents CPP-ACP and NaF.

Therefore, the aim of this study was to determine the effect of the CPP-ACP paste, NaF, and a combined application of CPP-ACP and NaF on MH of artificial carious enamel.

MATERIALS AND METHODS

Samples Preparation:

Fifty sound human maxillary premolars extracted for orthodontic purpose were collected, All teeth were stored in physiological saline after extraction at 37^{o}C until their use. The roots discarded, and crowns sectioned longitudinally in to two pieces, in the mesiodistal direction using mintom (Struers, Denmark). Then, the buccal portion of specimens were embedded in acrylic resin with outer buccal surface exposed. The enamel surfaces were ground wet using 600-2000 grit silicon carbide abrasive paper to expose standardized flat enamel surfaces for microhardness. Each specimen was then coated under digital stereomicroscope (X 40) with two layers of acid resistance nail varnish, leaving 3×3mm window on the middle third of the enamel surface. [3,16,17] After that, specimens randomly divided into 5 groups with 10 specimens in each group as follows:

Group A: Specimens stored in artificial saliva, the composition of artificial saliva was (0.058 ppm fluoride, 1.55 mmol calcium and 0.92 mmol phosphate, pH 7). [18] (control +ve)

Group B: Specimens were demineralized (discus later) and stored in artificial saliva (control –ve).

Group C: Specimens were demineralized and, then treated with CPP-ACP paste (containing Pure water, glycerol, CPP-ACP, D-sorbitol, CMC-Na, propylene glycol, silicon dioxide, titanium dioxide, xylitol, phosphoric acid, flavoring, zinc oxide, sodium saccharin, ethyl p-hydroxybenzoate, maganisium oxide, guar gum, propyl p-hydroxybenzoate, butyl p-hydroxybenzoate) (GC Tooth Mouse, GC CORPORATION, Tokyo, Japan) and stored in artificial saliva.

Group D: Similar to group C, but the treatment agent was topical natural NaF solution (containing 2% sodium fluoride and 0.9% fluoride ion) (NUPRO, DENTSPLY, U.S.A.).

Group E: Similar to group C, but the treatment agent was a mixture of CPP-ACP and NaF in a ratio of (2:1). The materials dispensed into glass slab using a syringe and then mixed at 29±2^{o}C for 1 minute until homogenous mixture was obtained.

Demineralization procedure:

Each specimen before any storage and treatment (in all groups except group A) was individually suspended in 20 ml of acidified buffered demineralizing solution for 2 weeks at temperature of 37^{o}C with constant stirring to create caries like lesions. The demineralized solution contained 2.2 mmol/L $CaCl_2$, 2.2 mmol/L NaH_2PO_4 and 50 mmol/L acetic acid adjusted to pH 4.5 with NaOH. The pH values of demineralization solution was measured every day using pH meter (HI 8014, HANNA instruments, Bioblock Scientific, Illkirch, France), and the demineralized solution was replaced every day. [3, 16,19]

Treatment Procedures:

Specimens were thoroughly rinsed with deionized water and delicately dried using a filter paper. Treatment procedure consisted of 5 minute application of treatment agent according to their group with aid of cotton tip then rinses thoroughly with deionized water for 1 minute. This procedure was performed once a day for 2 weeks. After treatment, the specimens were stored at 37^{o}C in the artificial saliva. While the group that was not subject to treatment as in groups A and B, the specimens were left in artificial saliva at 37^oC for 2 weeks. Artificial saliva was replaced every day, and it pH value was measured every day using digital pH meter (J, Morita Corporation, Japan). [5]

pH Cycle:

A pH cycling regimen included alternative demineralization (three hours) and remineralization (21 hours) for two weeks. For the demineralization phase, the demineralization solution used for the induction of enamel lesions was used, and for the remineralization phase, a solution composed of the following was used: CaCL2 1.5 mmol/L, NaH_2PO_4 0.9 mmol/L, KCl 0.15 mmol/L and adjusted at pH 8. The specimens were stored during each phase of pH cycle at 37^oC with constant stirring. The pH values of demineralization and remineralization solutions were measured every day using pH meter. Also the demineralization and remineralization solutions were replaced every day. [3,4,5,12,14,16,19]

Microhardness Assessment:

The surface microhardness (SMH) of the specimens was determined using Vickers microhardness testing machine (OLPERT, Germany) with a Vickers diamond indenter and scaled microscope (ZEISS, Germany) as shown in Figure (1). A load of 50 g was applied to the surface of the specimens for 15 seconds. Five indentations were equally placed over a circle of 1 mm diameter at the middle third of the specimens. The diagonal length of the indentations was measured by scaled microscope (20 X lens) and Vickers values converted into microhardness values. MH was obtained using the following equation: HV=1.854 P/d where HV is a Vicker hardness in Kgf/mm^2 (Mpa), P is the load in Kgf and d is the length of diagonal in mm. [3,4]

Statistical Analysis:

One way analysis of variance and Tukey post Hoc multiple range tests ($P \leq 0.05$) were performed to evaluate the differences on MH among tested groups.

RESULTS

One way analysis of variance demonstrated significant differences on the SMH among tested groups as shown in Table (1).

Tukey test revealed that MH for control +ve group and CPP-ACP mixed with NaF treated group not significantly different for each other but both were significantly higher than control –ve group and those groups treated with CPP-ACP or NaF alone. CPP-ACP treated group and group treated with NaF had SMH significantly higher than control –ve group, while significantly there was no difference between them. As shown in Table (2).

DISCUSSION

Minerals, primarily calcium and phosphate, leak out from the hydroxyapatite crystals during demineralization and in situations where demineralization outpaces remineralization, this leads to the development of subsurface lesions. These initially involve only the enamel and often result in the appearance of white spots where sufficient subsurface mineral content has been lost to alter the optical properties of the dental hard tissues. [3] Considering the importance of the surface layer in caries progression, the evaluation of changes in this region is relevant. MH measurement is a suitable technique for this purpose and appropriate for a material having fine microstructure, non-homogenous like enamel. As well as, MH indentation provides a relatively simple, non-destructive and rapid method in demineralization and remineralization studies. It had been reported a good correlation between enamel microhardness and mineral loss in caries lesions. [3,4,14,15,16,17]

Flat and polished enamel specimens were used in the present study in an attempt to standardize specimens and remove natural variations in surface enamel between teeth and between different tooth sites and types, which may result in different responses to acid dissolution. [20] However, it should be noted that natural tooth surfaces erode more slowly than polished surfaces. [21] In this study, CPP-ACP used in combined with NaF in a ratio of 2:1 this ratio of combination was used because CPP-ACP tooth mouse used in this study not contain fluoride, therefore in an attempt to increase the effectiveness in enamel remineralization and MH addition of fluoride to the paste of CPP-ACP tooth mouse was done. The time of the treatment used in the study is about 5 minute once a day for 2 weeks, this result from the idea that increase the time and frequency of application of the treatment agents would lead to increase the precipitation of the minerals content in the treatment agents in the enamel surface and this would lead to increase the remineralization and as a result increase the MH of enamel. pH-cycling systems used in this study, because it had been shown by previous studies that its beneficial for evaluation caries lesion and mineral changes in

dental hard tissues. As well as, the best way to simulate the environment in vitro is to apply pH-cycling models which is equivalent to dynamics dental decay. (3,4,5,12,14,16)
A load of 50-gram was chosen in this study, because indentations made with this load have previously been shown to be a sensitive measure of mineral changes during microhardness testing. (3,4) Also it was shown in this study when a load used higher than 50 gram it will lead to crack into enamel surface.

The results of this study show that there was a significance differences on microhardness of groups treated either with CPP-ACP or NaF from control –ve group. Recently casein phosphopeptideamorphous calcium phosphate (CPP-ACP) derived from cow's milk has been reported to reduce the demineralization process of the tooth structure and enhance the remineralization process. 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. (8,9,10,11,22,23) However, the role of topical fluoride in caries prevention and enhancing tooth mineral resistance to a cariogenic challenge is well known. The mechanism for reducing demineralization and facilitating remineralization involves modification of the mineral structure of enamel with creation of fluoridated calcium and phosphate mineral phases, and increasing the surface enamel fluoride content. On the other hand, fluoride is the most commonly used remineralizing agent. When the acid attacks the enamel surface, the pH begins to rise and fluoride present in the microenvironment causes enamel dissolution to stop. As the pH rises, new and larger crystals that contain more fluoride (fluorhydroxyapatite) form, thereby, reducing the enamel demineralization by forming fluorhydroxyapatite crystals and enhancing remineralization. Normally, remineralization by fluoride is a self-limiting surface phenomenon that prevents penetration of ions into the depth of the lesion. Rapid deposition of fluorapatite forms a firm surface layer, which is more resistant to further demineralization. (3,4,24,24) Therefore, because of the previous explained reasons, it was found in this study that CPP-ACP and NaF would result in increasing the microhardness of the demineralized enamel surface. This finding in this study was in agreement with the findings of several previous studies (3,4,14,15,16,17,25).

In this study, microhardness results show that teeth treated with CPP-ACP mixed with NaF have significantly more hardness than those groups treated either with NaF or with CPP-ACP alone. The anticaries role of CPP-ACP is related to its containing calcium and phosphate, and the negative relationship between caries and the presence of calcium and phosphate has been generally accepted. At the same time, the fluoride ion has been shown to reduce the speed of demineralization and enhance the reproduction of enamel crystals. When CPP-ACP is combined with fluoride toothpaste, the fluoride ions react with CPP-ACP to form casein phosphopeptide–amorphous calcium fluoride phosphate. It had been established by previous studies that the nanocomplex, casein phosphopeptide–amorphous calcium fluoride phosphate, provided calcium, phosphate, and fluoride ions to the surface of the teeth and therefore had a tremendous effect on enamel remineralization. (5,9,12,13) Therefore, it can be explain because of these before discus causes why the findings of the current study proved the combined benefits of CPP-ACP and NaF in enhancing the microhardness of the demineralized enamel more than if the CPP-ACP or NaF was used alone. The results of our experiment are coordinate with several previous studies.(3,24,26)

CONCLUSIONS

Within the limitation of this study the following conclusions can be drawn:

1. NaF, CPP-ACP, and combination of (NaF, CPP-ACP) were significantly increased the microhardness of the demineralized enamel surface regarding of application technique.
2. NaF and CPP-ACP show similar effect on enamel microhardness.
3. CPP-ACP and NaF showed higher microhardness value than individual materials.

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Figure (1): (A): Specimen fixed on Vicker microhardness testing machine.
(B): Specimen fixed on scaled microscope.

Table (1): One way analysis of variance for the differences on microhardness among tested groups

	Sum of Squares	Degree of Freedom	Mean Squares	F-value	P-value*
Between Group	71985.720	4	17996.421	592.425	0.000
Within Groups	1367.000	45	30.378		
Total	73352.720	49			

***P≤0.01 highly significant different exist.**

Table (2): Tukey test for the differences on microhardness among tested groups

Tested Groups	Mean(Mpa)±SD
Control +ve	277.3±5.03 A*
Control –ve	171.0±5.47 C
CPP-ACP	234.1±5.46 B
NaF	227.0±5.30 B
CPP-ACP &NaF	270.6±5.44 A

***The different letters vertically mean significant difference exist.**