The Effect of Topical Remineralization Agents on Surface Microhardness of Enamel (ex vivo research)

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Abstract

Demineralization and remineralization of dental enamel cause changes in the surface microhardness. Topical remineralization agents with calcium-phosphate technology in the form of varnish can be used to improve enamel remineralization. The aim of this study was to quantify the surface microhardness in demineralized enamel after application of remineralization agents containing sodium fluoride (NaF) with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), functionalized tri-calcium phosphate (f-TCP) or 5% calcium fluoride.

A total of 30 human sound maxillary premolars were flattened and polished. The enamel surfaces were demineralized with 37% phosphoric acid. The samples were randomly divided into three groups. Each group then received one of the following treatments: remineralization agents containing NaF with CPP-ACP (MI varnish, GC Asia, Japan), f-TCP (Clinpro varnish, 3M, USA), or 5% calcium fluoride (Bifluorid 10 varnish, VOCO, Germany). All samples were immersed in artificial saliva and stored at 37°C. The microhardness of enamel was measured after 7 and 14 days using Vickers test.

One-way ANOVA and post-hoc Tukey test results showed significant differences between groups after remineralization on day 7 and 14 ($p \leq 0.05$). The GLM Repeated Measure ANOVA test results showed significant differences between demineralization and remineralization on day 7 and 14. CPP-ACP group showed the highest increase in enamel microhardness (214,56±4,29 and 236,53±2,14 VHN) compared to f-TCP group (229,77±8,13 and 221,53±9,48 VHN) and calcium fluoride 5% group (219,33±7,11 and 220,34±5,02 VHN).

CPP-ACP group is the best remineralization agent compared to f-TCP and calcium fluoride 5% group.

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Introduction

Demineralization is the initial process of tooth decay as a result from carbohydrate fermentation by both oral bacterias and others (such as dental erosion, dental whitening, and drugs).[1] Furthermore, the most vulnerable aspect of dental enamel is hydroxyapatite; which contains calcium and phosphate. Demineralization starts as soon as the oral pH reaches critical pH (pH 5). The reaction between hydrogen ions (H^+) and phosphate ions (PO_4^{3-}) leads to dissolution of hydroxyapatite crystals. Demineralization and remineralization are dynamic processes and ionic imbalance may influence these processes. Hence, if the demineralization process is more dominant, it may cause structural change in the enamel (microhardness, strength, calcium and phospate, fluor).[2]

Related to demineralization process, the enamel dissolution can be repaired with natural remineralization process or remineralization agents. Hydrogen ions that have been dissolved in demineralization process is replaced with fluoride ions in saliva to form fluor apatite ($Ca_{10}(PO_4)_6F_2$). Fluor apatite is more resistant to

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acid due to limited deposit of fluor in saliva so remineralization is restricted only on superficial enamel. It is crucial to administer additional remineralization agent containing calcium-phospate and fluor.[3–5]

There are various remineralization agents available in market. The well-known remineralization agent is topical fluor or fluor varnishes. The method of administration can be done in two ways: personal (pastes, gargle solution) and professional (varnish, gel).[6] Topical fluor varnish acts as a fluor reservoir in oral cavity. Thus, it can enhance remineralization.[7]

Fluor varnish available in public is 5% sodium fluoride combined with the casein phosphopeptide- amorphous calcium phosphate (CPP-ACP), functionalized tri-calcium phosphate (f-TCP), or 5% calcium fluoride, 5% sodium fluoride. Such remineralization agents have been briefly studied by many, but their effects in enamel remineralization need further study. It has been reported that varnish containing tri-calcium phospate had increased enamel hardness over 2 weeks of application.[8] On the other hand, the other study reported that CPP-ACP treated enamel showed increased enamel hardness, compared to f-TCP.[3,9,10] The aim of this study was to compare the surface microhardness in demineralized enamel after application of remineralization agents containing 5% sodium fluoride with CPP-ACP, f-TCP or 5% calcium fluoride after 7 and 14 days.

Materials and methods

Thirty sound human maxillary premolars are decoronated using a disc bur (Isomet, Buehler Inc., USA) with water spray. Each samples then placed on a PVC mold and embedded with acrylic resin. The uncovered buccal aspects of each samples were smoothed and flattened semi automatically (LaboPol-21, Struers, Denmark) using 600 and 2000-grit silicone carbide paper (Nikken, Japan). Next, the samples were polished using alumino silicate paste (1µm) to create flat and smooth surfaces.

The flat and smooth surfaces of each sample was subjected to demineralization using 37% phosphoric acid (Ultra-Etch, Ultradent, USA) for thirty seconds and washed with sterile water then dried using air syringe. Initial enamel microhardness after demineralization protocol was measured using Vickers Microhardness Tester (Tester Shimadzu HMV-G21DT, Japan). Microhardness test was carried out in three indentations on buccal smooth surface of each tooth (Baseline value). Measurement was done under microscope with 40x magnification. The indentation's load was 50 gf for 15 seconds.

Samples were randomly divided into 3 experimental groups (n=10). Each group was exposed to CPP-ACP and 5% sodium fluoride (MI varnish, GC Asia, Japan), f-TCP and 5% sodium fluoride (Clinpro varnish, 3M, USA) and 5% calcium fluoride varnish + 5% sodium fluoride (Bifluorid 10 varnish, VOCO, Germany) for 1 minute, distinctly. They were immersed in artificial saliva and stored in an incubator (Heratherm, Germany) with a temperature of 37°C. Artificial saliva was replaced every 24 hour. Enamel microhardness was evaluated on day 7 and day 14 using a Vickers Microhardness Tester (Tester Shimadzu HMV-G21DT, Japan).

The differences in the microhardness values of the various groups were compared parametrically by One-way analysis of variance (ANOVA). The GLM Repeated Measured ANOVA test was used to analyze the microhardness after application of varnish by comparing the baseline to samples on day 7 and day 14. Data analysis was performed using IBM SPSS Statistics 20 software (SPSS Inc, Chicago, IL, USA).

This research was conducted in accordance with all the provisions of the local human subjects oversight committees guidelines and policies of the Research Ethics Committees Faculty of Dentistry, Trisakti University (No: 133/S2-Sp/KEPK/FKG/7/2018).

Results

One-way ANOVA showed that there was a significant difference between the test groups on day 7 and 14. The Means, standard deviations and statistical results of enamel microhardness value are provided in Table 1.

Tukey post-hoc test (Table 2) revealed significant differences in the day 7 of remineralization between f-TCP group compared to CPP-ACP and 5% calcium fluoride group ($p \leq 0.05$), but there was no significant difference between CPP-ACP and 5% calcium fluoride group ($p > 0.05$). Day 14 remineralization showed significant differences between f-TCP and CPP-ACP group, and between CPP-ACP and 5% calcium fluoride group ($p \leq 0.05$).

Varnish Group	Application time	Mean ± SD (VHN)	P
CPP-ACP	Baseline	153.47 ± 4.14	0.13
	on day 7	214.56 ± 4.29	<0.001*
	on day 14	236.53 ± 2.14	<0.001*
f-TCP	Baseline	156.30 ±1.39	
	on day 7	229.77 ± 8.13	
	on day 14	221.53 ± 9.48	
5% calcium fluoride	Baseline	152.67 ± 5.65	
	on day 7	219.33 ± 7.11	
	on day 14	220.34 ± 5.02	

Table 1. Means ($\bar{x}$) and Deviations (SD) of Enamel Microhardness Value.
*One-way ANOVA ($p \leq 0.05$)

Varnish Group		P
Day 7 remineralization		
f-TCP	CPP-ACP	<0.001*
	5% calcium fluoride	0.005*
CPP-ACP	5% calcium fluoride	0.36
Day 14 remineralization		
f-TCP	CPP-ACP	<0.001*
	5% calcium fluoride	1
CPP-ACP	5% calcium fluoride	<0.001*

Table 2. Significance between Groups on 7th and 14th after Varnish Application.
*Tukey post hoc test ($p \leq 0.05$)

Varnish Group		p
f-TCP (Clinpro varnish)		
Baseline	Remineralization day 7	<0.001*
	Remineralization day 14	<0.001*
Remineralization day 7	Remineralization day14	0.1
CPP-ACP (MI varnish)		
Baseline	Remineralization day 7	<0.001*
	Remineralization day 14	<0.001*
Remineralization day 7	Remineralization day 14	<0.001*
5% calcium fluoride (Bifluorid 10 varnish)		
Baseline	Remineralization day 7	<0.001*
	Remineralization day 14	<0.001*
Remineralization day 7	Remineralization day 14	1

Table 3. Significance of Enamel Microhardness Value for each Group on 7th and 14th days after Varnish Application.
*GLM repeated measure ANOVA ($p \leq 0.05$)

The GLM Repeated Measure ANOVA test asserted a significant increase in enamel hardness value in f-TCP and 5% calcium fluoride varnish group from baseline to day 7 and 14 after application ($p \leq 0.05$), but there was no significant difference in enamel hardness in day 7 and 14 ($p>0.05$). F-TCP group showed that the enamel microhardness is decreased on day 7 (229.77±8.13 VHN) compared to day 14 (221.53±9.48 VHN). There was also a significant increase in enamel microhardness value in CPP-ACP varnish group from baseline (153.47±4.14 VHN) to day 7 (214.56±4.29 VHN) and day 14 (236.53±2.14 VHN). The results are presented in Table 3 and enamel microhardness values for each group are shown in Figure 1.

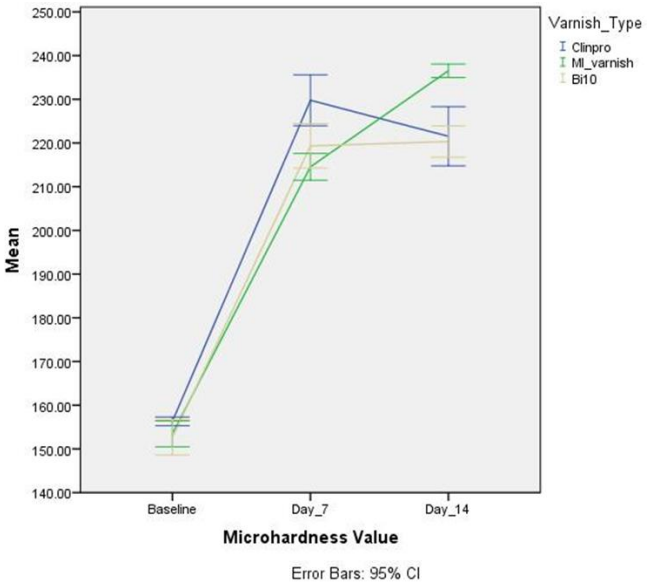

Figure 1. Enamel Microhardness Values.

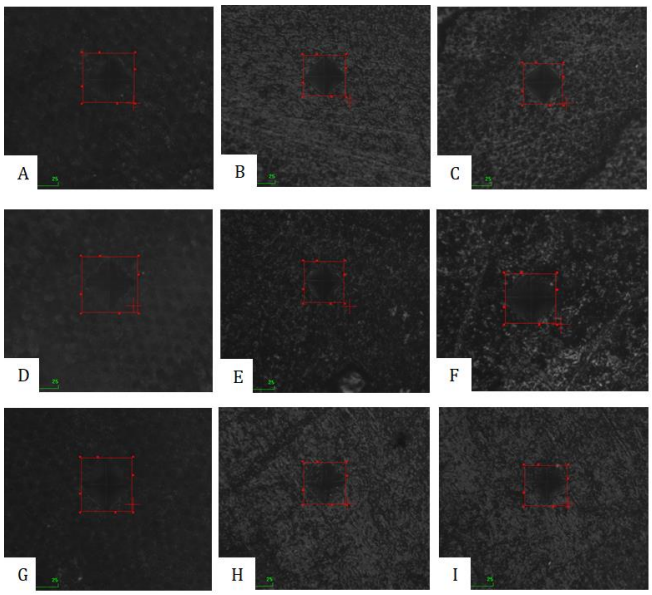

Figure 2. Image of enamel surface after indentantion using Vickers microhardness tester: A. Baseline (CPP-ACP varnish); B. After 7 days (CPP-ACP varnish); C. After 14 days (CPP-ACP varnish); D. Baseline (f-TCP varnish); E. After 7 days (f-TCP varnish); F. After 14 days (f-TCP varnish); G. Baseline (5% calcium fluoride varnish); H. After 7 days (5% calcium fluoride varnish); I. After 14 days (5% calcium fluoride varnish).

Discussion

The result of this study is all varnish groups increase the enamel microhardness value, although only CPP-ACP group had a significant increase. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is a compound responsible for stabilizing calcium, phospate, and fluoride ions.[5] Based on the result of this study, the CPP-ACP varnish group had a superior remineralization effect on enamel compared to other groups. This explains that the CPP–ACP component, the amorphous calcium phosphate, is able to transform the unsaturated calcium into saturated calcium. Furthermore, its nanocomplex particle size is able to penetrate into the porosity of enamel surface and significantly release calcium ions and inorganic phosphate at a constant rate. The CPP-ACP may also prevent demineralization by forming crystalline layer, filling interprismatic enamel, and blocking enamel prisms.[7,11,12] This research showed that the CPP-ACP varnish experimental group had more homogenic enamel structure than other groups. These findings can be observed by 40x magnification microscopic image on Vickers Microhardness Tester (Figure 2).

In this study, the f-TCP varnish group insignificantly decrease hardness value on day 7 and 14. These findings might be affected by low discharge of calcium and anorganic phosphate ion, low ratio of functionalized tri-calcium phosphate (f-TCP) in the product, and low solubility of tri-calcium phosphate.[7] Tricalcium phosphate (TCP) is a type of calcium phosphate based delivery system. In this system, tricalcium phosphate is functionalized (f-TCP) with sodium lauryl sulphate and 5% sodium fluoride. As the mineralization agent makes contact with saliva, it releases calcium and fluoride, thus enhancing mineralization process and prevent demineralization for a long time.[3,8]

According to this study, 5% calcium fluriode group (Bifluorid 10 varnish) also could increase the enamel microhardness value from initial application until day 7 and 14, although there was no significant increase after day 7. Adjuvant with the previous study, the CPP-ACP varnish possesses a good remineralization properties compared to f-TCP,[10] and a remineralization ability superior to other varnishes.[9] Currently there are only a few studies on 5% calcium fluoride varnish, which are probably due to its new release on the market. The research on 5% calcium fluoride varnish revealed that this material has a good remineralization property, although it is still lower than that of MI varnish. Lower mineralization effect may be altered by its high viscosity .[13,14]

These three experimental groups showed good remineralization abilities on day 7 and 14 because they increased enamel microhardness. Increased enamel microhardness resulted from fluoride ions intake, as well as other ions such as calcium and phosphate. A reservoir layer is formed on the enamel due to high ionic concentration, creating a strong and stable enamel crystal.[3] Remineralization can also occur secondary to ionic reservoir in saliva[8,15] that could alter the result of this study. Clinically, these remineralization agents are useful as calcium and phosphate ions reservoir and can be used widely as remineralization materials.

During the experimental procedure, all samples were immersed in the artificial saliva which was regularly replaced every 24 hours. Samples were also kept safely inside an incubator (37^0C) during the remineralization process. These procedures were done to ensure a constant temperature and humidity, as well as creating an oral cavity-like environment.[16] Saliva also contained crucial ions responsible for remineralization such as potassium chloride, magnesium chloride, potassium hydrogen phosphate, and potassium dihydrogen phosphate.[17] The use of phosphoric acid 37% is also resembling demineralization process in early carious lesion.[18]

Microhardness test is utilized to evaluate the tooth's physical strength. Hardness test is strongly related to demineralization and remineralization processes that occur in tooth enamel structure. Demineralization process is responsible for releasing calcium and phosphate ions from hydroxyapatite crystal. Release of such ions is linearly related to enamel hardness.[19] Vickers Microhardness Tester was used in this study. This method was selected because of its feasibility and it only required a little surface to be tested. Thus, it is easier to measure the indentation area. The method of measurement encompasses pressing on the tooth surface on a specific load and time, then indentation area is measured diagonally with optical microscope. Value of microhardness can be obtained by adding diagonally indentation areas.[20] Value of

load and time used in this study were respectively 50 gf and 15 seconds. These values represent normal tooth load.[21]

Conclusions

These three remineralization agents can be used as topical fluor varnishes containing calcium-phosphate technology to promote remineralization of dental enamel. Casein phosphopeptide-amorphous calcium phosphate (MI varnish) showed greater increase of enamel hardness and stability compared to f-TCP and 5% calcium fluoride varnish.

Declaration of Interest

The authors declare no conflict of interest with respect to publication of this article.

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