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Efficacy of Functionalized Tri-Calcium Phosphate Containing Fluoride Varnish for Enamel Remineralization after Exposure to pH cycling regimen

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Abstract

Background: Remineralization of early carious lesions is a main concern in conservative dentistry. Many remineralizing agents were introduced in the market. Thus this study aimed to evaluate the effectivness of two remineralizing varnishes on the micro-hardness and ultra-morphology of deminaralized enamel. **Methods**: Human anterior teeth were subjected to pH cycling regimen for 5 consecutive days. Specimens were divided into two groups (G) according to applied varnishes: GI: fluoride varnish (Duraphat), GII: functionalized tri-calcium phosphate (F-TCP) containing fluoride varnish (Clinpro™-White). Varnishes were applied for three minutes per day for seven consecutive days. After each application procedure, the specimens were stored in artificial saliva at 37°C. Micro-hardness and ultra-morphological evaluation were tested for untreated, demineralized and remineralized enamel. **Results**: Mann-Whitney U test revealed significant increase on % of change of Vickers hardness number (VHN) after the remineralization process (*Mdn*= -17.81%) for Duraphat and Clinpro™-White Varnishes (*Mdn*= -4.68%). Also, there was insignificant difference between the % of change of VHN Duraphat (*Mdn*= -17.81%) and Clinpro™-White Varnishes (*Mdn*= -4.68%) after remineralization at p=0.1. SEM photomicrographs showed smooth enamel surface for untreated enamel specimens. Demineralized enamel showed a honeycomb like structure. Duraphat treated enamel, showed some of the interprism cavities were being filled with relative decrease of their depth. Clinpro™-white treated enamel showed globular structure deposits filling the interprismatic spaces. **Conclusion**: F combination with F-TCP provides same remineralization potential regarding enamel micro-hardness, with no improvement in the surface morphology of demineralized enamel surfaces compared to the fluoride varnish alone.

Key words: Remineralization; Fluoride; Tri-calcium phosphate.

Introduction

Under normal physiological conditions (pH 7) saliva is supersaturated with calcium and phosphate ions, making caries progress slow. When the pH falls to 4.5 -5.5 due to acids produced by bacteria after sugar consumption, the saturation point of the minerals in the surrounding fluid is changed. The critical pH of hydroxyapatite is around 5.5. Below the critical pH, teeth demineralization occurs while above the critical pH, remineralization occurs [1, 2].

Therefore, having sufficient levels of bioavailable calcium, phosphate and hydroxide or fluoride ions in the oral fluid bathing the teeth is essential [2,3].

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Many of these ions have been included into creams, dentifrices, restorative materials and more recently into varnishes. The aim of introducing fluoride varnishes was to provide an additional intraoral reservoir of fluoride ions by the formation of calcium fluoride (CaF_2), 'calcium fluoride-like' or biologically/bacterially bound calcium fluoride (CaF^+). It was confirmed that calcium ion and fluoride ion availability limit the formation of these reservoirs [3, 4]. Thus, some manufacturers included calcium and inorganic phosphate ions into fluoride varnishes in an attempt to increase the retention of fluoride and calcium ions in the oral environment and further improve the efficacy of remineralization of early lesions. Many technologies were used for adding calcium and phosphate ions into fluoride remineralizing agents. One of the recent technologies was the crystalline; such as functionalized tri-calcium phosphate (F-TCP) [5].

Thus; it was interesting to evaluate the effectiveness of F-TCP containing fluoride varnishes on enamel micro-hardness and ultra-morphology after exposure to pH cycling regimen, compared to a fluoride varnish. The null hypothesis was that there would be no significant differences in enamel micro-hardness and ultra-morphology from the different varnishes.

Materials and Methods

Materials selection

Two commercial remineralizing varnishes were tested in this study; fluoride varnish (Duraphat varnish) and functionalized tri-calcium phosphate (F-TCP) containing fluoride varnish (Clinpro™-White Varnish). Materials name, composition and manufacturers are represented in Table 1.

Table 1: Materials name, composition and manufacturers

Product	Composition (w/w)	Manufacturer
Duraphat Varnish (Fluoride varnish)	30–60% colophonium, 10–30% ethanol, 5% sodium fluoride, other ingredients	Colgate-Palmolive, New York, USA
Clinpro™ White Varnish (F-TCP containing fluoride varnish)	30–75% pentaerythritol glycerol ester of colophony resin, 10–15% n-hexane, 1–15% ethyl alcohol, 1–5% sodium fluoride, 1–5% flavour enhancer, 1–5% thickener, 1–5% food grade flavour, <5% modified tricalcium phosphate	3M ESPE, USA

Teeth selection

Thirty human maxillary central incisors scheduled for extraction for periodontal reasons were included in this study. Teeth were cleaned under running water polished with

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pumice to get rid of debris and stains from enamel surface. The teeth were then stored in phosphate buffer solution (g/L): [(Na_2HPO_4 (0.578), KH_2PO_4 (0.353) dissolved in distilled water containing 0.02% sodium azide adjusted at PH=7] and stored at 4˚C for a maximum period of one month before being used [6].

Specimens' preparation

Roots of the central incisors were removed by sectioning the teeth at the cement-enamel junction using diamond disc (Edental Golden S.A.W., Switzerland) mounted in a low-speed hand piece under copious water coolant. The pulpal tissues were removed using endodontic file, then the root canals openings were sealed with wax. Each crown segment was embedded in a self-cured acrylic resin with the labial surface of the tooth directed outward. Labial enamel surfaces were ground using SiC paper-grit #80 and polished using #600, #1200 and #2400-grit SiC papers to obtain flat enamel surfaces [6,7]. A total of 30 specimens were prepared and kept in artificial saliva at 37°C for 24 hours before testing [8]. Baseline micro-hardness testing was carried out for all the thirty specimens using Vickers micro-hardness tester.

Demineralization process of the specimens

Following testing the specimens for baseline micro-hardness, all specimens were subjected to pH cycling regimen including alternate demineralization (3 hours in demineralizing solution) and remineralization (21 hours in artificial saliva) for 5 consecutive days. Specimens were washed in distilled water at each change of medium. The demineralization solution and the artificial saliva were changed every 24 hours [9].

The saliva composition used as storage medium was: 1.5 $mmolL^{-1}$ $Ca[NO_3]_2.4H_2O$, 0.9 $mmolL^{-1}$ $NaH_2PO_4.2\ H_2O$, 150 $mmolL^{-1}$KCl, 0.1 $molL^{-1}$ Tris buffer, 0.03 ppm F. The saliva pH was adjusted and maintained at 6.57 [10].

The composition of the demineralization solution was as follows: 2.2 mM calcium chloride ($CaCl_2\ 2H_2O$); 2.2 mM monosodium phosphate ($NaH_2PO_4\ _7H_2O$), 0.05 mM lactic acid; pH was adjusted to 4.5 with 50% sodium hydroxide (NaOH) [11]. All the 30 demineralized specimens were subjected again for micro-hardness testing.

Application of remineralizing varnishes

The 30 demineralized specimens were arbitrarily divided into two groups (n=15) according to the different remineralizing varnishes tested.

Group I: received Duraphat varnish

Group II: received Clinpro™-White Varnish

The specimens were rinsed with distilled water then dried with cotton, and then the remineralizing varnishes were applied for three minutes per day, for seven consecutive days [10]. After each application procedure, the specimens were stored in artificial saliva at 37°C. At the end of the seven days the remineralized specimens were subjected to micro-hardness testing.

Micro-hardness testing

The enamel micro-hardness testing was performed using Vickers micro-hardness tester (Shimadzu HMV-M Micro-hardness tester; Newage Testing instruments Inc., Southampton, PA, USA) under 200gm load, and 15 seconds dwell time [11]. The loaded diamond was allowed to sink and rest on the enamel surface for 15 seconds and the Vickers

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hardness number (VHN) was determined. Three indentations were performed on each specimen, and the mean value of the readings was calculated and recorded. Micro-hardness measurements were obtained at baseline (before enamel demineralization), after demineralization process and after remineralization process.

Ultra-morphological evaluation

For evaluation of morphological changes occurring in specimens after each treatment protocol, scanning electron microscope (SEM) (Model Quanta 250 FEG) was used. Representative specimens from each group were mounted on aluminum stubs, dried in vacuum, then sputter coated with gold (Ladd sputter coater, USA). Photomicrographs were taken at 2000X magnifications for; untreated enamel surface, enamel specimens after demineralization and enamel specimens after treatment with each remineralizing varnish.

Statistical analysis

Data presented as Median (Mdn), standard error (SE), minimum and maximum values of percentage of change in micro-hardness (VHN) for the difference between procedure and application of remineralizing varnishes. A nonparametric test (Mann–Whitney U test) have been used to study the difference between procedures and the difference between the remineralizing varnishes on mean % change in VHN. Statistical analysis was performed with IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics Version 20 for Windows.

Results

Median (*Mdn*), standard error (SE), minimum and maximum values of percentage of change in micro-hardness (VHN) for the difference between procedure and application of remineralizing varnishes are presented in Table 2.

Table 2: Median, standard error (SE), minimum and maximum values of percentage of change in micro-hardness (VHN) for the difference between procedure and application of remineralizing agent

			Demineralization process	**Remineralization process**	p-value
			% of change in VHN		
Remineralizing varnishes	**Duraphat varnish**	Median	-40.97	-17.81	0.05*
		SE	0.46	2.01	
		Minimum	-41.91	-18.41	
		Maximum	-40.34	-12.10	
	Clinpro™ White Varnish	Median	-40.68	-4.68	0.05*
		SE	0.30	0.42	
		Minimum	-40.82	-5.10	
		Maximum	-39.85	-3.70	
p-value			0.4 NS	0.1 NS	

*significant different at p≤0.05. *= Significant;* NS; non-significant

SEM photomicrographs showed smooth enamel surface for baseline untreated enamel specimens (Figure.1a). on the other hand; photomicrographs for demineralized enamel showed a typical prismatic structure, the etching process involves the inner areas of the prism creating a honeycomb like structure as represented by (Figure.1b).

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Photomicrographs of enamel surface remineralized with Duraphat varnish, shows some of the interprism cavities were being filled with relative decrease of their depth (Figure.1c). Demineralized enamel surfaces treated with Clinpro™-white varnish showed deposits filling the interprismatic spaces forming a globular structure over the demineralized enamel surface (Figure.1d).

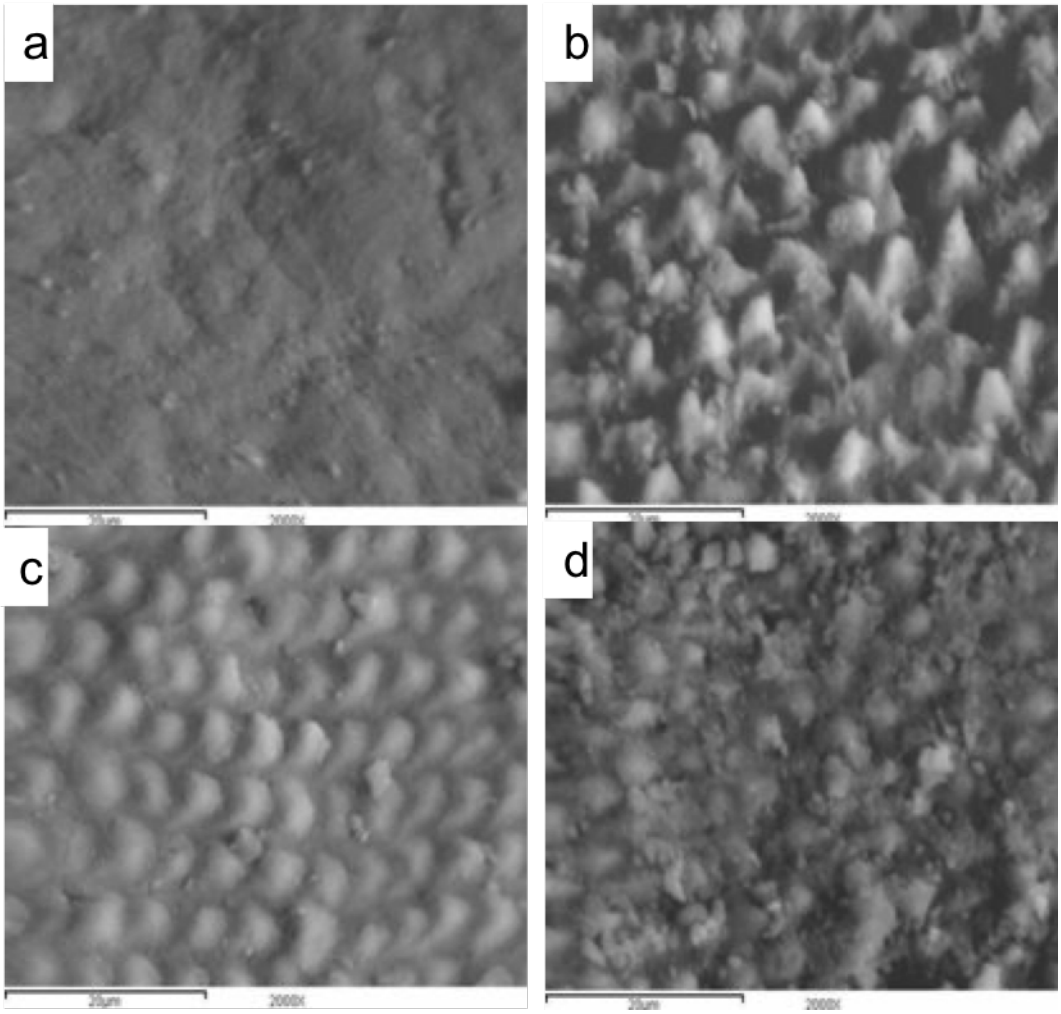

Figure.1. Photomicrographs 2000X a. untreated enamel surface (control), b. Demineralized enamel surface, c. Remineralized enamel using Duraphat varnish d. Remineralized enamel using Clinpro™-White Varnish

Discussion

Both systemic and topical application of fluoride play an important role in caries prevention and resistance. Rapid loss of soluble fluoride from the teeth surfaces is the main disadvantage of topical fluoride application. To overcome this disadvantage manufacturers introduced recent technologies to improve the effect of fluoride varnish [5].

In this study fluoride and F-TCP fluoride varnishes were evaluated in term of their effect on demineralized enamel micro-hardness and ultra-morphology. Vickers hardness number (VHN) was selected over Knoop′s hardness number because the diamond shape indent obtained in VHN is easier and more precise to measure [12, 13]. In the present study, the enamel micro -hardness values were measured in three steps; baseline, after demineralization, and

remineralization. The average hardness value of baseline enamel obtained in this study was within the range reported in literature which ranges from 250 to 360 VHN [12]. Periods of demineralization in the pH cycling phase was three hours to simulate low cariogenic challenge that occurs in the oral cavity [9].

Results of this study revealed that there was significant increase on % of change of VHN after the remineralization process with both Duraphat and Clinpro™-White Varnishes compared to the demineralized specimens. This might be due to that both varnishes contain fluoride in their composition. To shift the balance from net demineralization to net remineralization three part per million (ppm) of fluoride in enamel are required. The anti-caries effect of fluoride comes from its capability to form fluoro-apatite, which is more acid-resistant than hydroxyapatite; the improvement of remineralization; interfering of ionic bonding during pellicle and plaque formation; and the inhibition of microbial growth and metabolism [14]. Increase the level of topical fluoride, fasten the minerals precipitation and closure of enamel surface pores, even if the subsurface areas are not remineralized [4].

Optimum concentration of fluoride with calcium phosphate was claimed by Clinpro™-White Varnish manufacturer which might help in remineralization of both enamel surface and subsurface. Clinpro™-White Varnish contains functionalized tri-calcium phosphate (F-TCP), which is formed by the solid-state ball milling of beta-tricalcium phosphate and sodium lauryl sulfate. Protecting the beta-tricalcium phosphate with sodium lauryl sulfate aimed to prevent the formation of calcium phosphate or calcium fluoride complexes upon application of F-TCP which would reduce remineralization by decreasing levels of available calcium and fluoride. This composition is hypothesized to form a “functionalized” calcium and a “free” phosphate that floats freely protecting the exposed calcium environs, thus prevent the calcium from rashly interacting with fluoride. When F-TCP is applied to saliva moistened tooth structure, it was supposed that the protective barrier breaks down making the calcium, phosphate, and fluoride ions free and accessible. The fluoride and calcium interact with the weakened enamel and provide a seed for enhanced remineralization [15].

The null hypothesis of this study was accepted as ClinPro™-white varnish showed non-statistically significant difference mean VHN recovery percentage compared to Duraphat varnish. This might be explained due to that both varnishes contain near amount of fluoride (1-5% by weight) and the amount of F-TCP being added to ClinPro™-white varnish was low to release sufficient amount of calcium and inorganic ions [16].

More over during the application of ClinPro™-White Varnish on enamel surface it showed high viscosity and greater amount of varnish was needed to cover the same surface than Duraphat varnish. This might lead to less contact and less diffusion of the varnish into the enamel surface [17]. This was confirmed by Clinpro™-White Varnish SEM photomicrograph which showed deposits filling interprismatic spaces forming globular structure over the demineralized enamel surface (Fig.1d).

Conclusion:

F-TCP containing fluoride varnishes provides same remineralization potential regarding enamel micro-hardness, with no improvement in the surface morphology of demineralized enamel surfaces compared to the fluoride varnish alone.

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Conflict of interest

The authors have no conflict of interest to disclose

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