Remineralization of white spot lesions by casein phosphopeptide amorphous calcium phosphate (CPP-ACP) and Tri calcium phosphate (TCP) versus fluoride with different application technique by measuring surface micro hardness and surface roughness:An *in vitro* comparative evaluation

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Article History: **Received:** 29-06-2023 **Revised:**16.07.2023 **Accepted:** 26.07.2023

Abstract:

Objective:

Effect of different techniques of application of CPP-ACP, TCP and fluoride by application varnish only then compared with varnish followed by paste on early white spot lesion.

Aim of Study

This study will be conducted to assess the effect of different remineralizing agents with different application technique a on enamel white spot lesion by micro hardness test, surface roughness

Conclusion

This study was conducted to assess the effect of different remineralizing agents with different application technique on enamel white spot lesion by micro hardness test, surface roughness.

Results :

The result of current study show that the highest microhardness recovery record by TCP Group either varnish or paste followed by CPP-ACP

DOI:10.48047/ecb/2023.12.11.61

Introduction

A paradigm shift is emerging in dentistry and dental treatments are now aimed at maximum conservation of tooth structure. Remineralization therapy is preferred in some cases. Many products as caries preventive materials are now available in the market which contains components that have the ability to initiate remineralization. **(Farooq, et al., 2013).**

Fluoride is the most commonly used remineralizing agent through formation fluorhydroxyapatite crystals with more resistant to acid attack and enhancing remineralization. Rapid deposition of fluorapatite forms a firm surface layer, which is more resistant to further demineralization **(Lata, et al., 2010)**.

A new remineralization technology based on phosphopeptide from milk protein casein has been developed. The casein phosphopeptides (CPP) contain multiphosphoseryl sequences with the ability to stabilize calcium phosphate in nanocomplexes, where CPP binds to amorphous calcium phosphate ACP in metastable solution preventing the dissolution of calcium and phosphate ions and acts as reservoir for supersaturate solution of bio-available calcium and phosphate thus facilitating remineralization. **(Lata, et al., 2010).**

Tri calcium phosphate (TCP) was recently introduced by 3M ESPE. This 1.1% NaF silica-containing paste contains an innovative functionalized tricalcium phosphate (fTCP) ingredient that, when evaluated in development.

Formulations, has been shown to boost remineralization performance relative to fluoride-only systems. The fTCP technology solves this problem by protecting its bioavailable calcium with a fluoride-repelling surfactant (sodium lauryl sulfate) an a result, can be readily combined in an aqueous dentifrice formulation with NaF **(Karlinsey, et al., 2010)**

It is expected that combination between remineralizing agents would enhanced remineralization compared to individual application. Therefore this study was conducted to assess the effect of different remineralizing agents with different application technique on enamel white spot lesion by micro hardness test, surface roughness.

Review of Literature

A review of studies investigated effects on early white spot lesion by using CPP-ACP, TCP and fluoride in form either paste or varnish including in-vitro as well in-vivo studies was performed. PubMed and Google Scholar between 2010 and 2016 were searched.

Oshiro, et al., 2007, used CPP–ACP paste on bovine teeth to demonstrate its remineralizing potential. Bovine teeth were cut into blocks. Few of the specimens were placed in lactic acid (demineralizing solution) and were then placed in artificial saliva. Remaining specimens were stored in CPP–ACP paste solution and they were then placed in demineralizing solution and artificial saliva. Scanning electron microscopy (SEM) was utilized to observe morphological features and it revealed that the specimens which were treated with CPP– ACP first, showed little morphological changes as compared to the remaining specimens and so it was concluded that CPP–ACP has the ability to prevent demineralizati

Materials and Methods :

Materials:

Three types of remineralizing agents available in two form either paste or varnish were used in this study. All Materials' specification, composition, lot number and manufacturers present in ***(table 1)***.

1.1. Remineralization using *Fluoride:*

a- DuraShield® CV Clear Varnish:

Fluoride Varnish is a topical dental varnish containing 5% sodium fluoride ***(Fig.1)***.

b- Colgate™ PreviDent®5000 plus Toothpaste :

This toothpaste is a Self-topical neutral fluoride dentifrice that containing 1.1% sodium fluoride and design to be used by both adults and pediatric patients ***(Fig.2)***.

1.2. Remineralization using CPP-ACP combined with fluoride:

a- MI Varnish™:

This varnish contains Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP), and its application leaves a film on tooth surfaces. It also contains 5% sodium fluoride varnish that has a desensitizing action when applied to tooth surfaces ***(Fig. 3)***.

b- MI Paste Plus™:

It is a water based paste containing CPP-ACP with fluoride; when applied in the oral environment, it bind to biofilms, plaque, bacteria, hydroxyapatite and soft tissue localizing bio-available calcium, phosphate and fluoride ***(Fig.4)***.

1.3. Remineralization using TCP combined with fluoride:

a- Clinpro™ White Varnish:

It is a solvent based white, viscous varnish that contains 5% sodium fluoride with tri-calcium phosphate ingredient; when it applied to the tooth surface, the solvent system (alcohol and water) evaporates rapidly, leaving

behind a hard lacquer-like film. This film adheres well to the surface of the teeth and slowly releases fluoride ion ***(Fig. 5)***.

b- Clinpro™ 5000 paste:

It is white toothpaste that contains 5000 ppm fluoride and tri-calcium phosphate ingredient. The product is intended to be used once daily in place of conventional toothpaste ***(Fig. 6)***.

1.4. Demineralizing solution:

The demineralizing solution had the following composition; $CaCl_2$ = 2.2 mM, NaH_2PO = 2.2 mM, Lactic acid = 0.05 M, Fluoride = 0.2 pp, adjusted with 50% NaOH to a pH 4.5***(Lata, et al., 2010)***.

1.5. Artificial saliva

The composition of the synthetic saliva is as follows: Na_3PO_4 - 3.90 mM $NaCl_2$ - 4.29 mM KCl - 17.98 mM $CaCl_2$ - 1.10 mM $MgCl_2$ - 0.08 mM H_2SO_4 - 0.50 mM $NaHCO_3$ - 3.27 mM, distilled water, and the pH was set at a level of 7.

2. Comar, et al., 2013)

Both artificial saliva and demineralizing solution were fresh prepared in Faculty of Pharmacy, Cairo University.

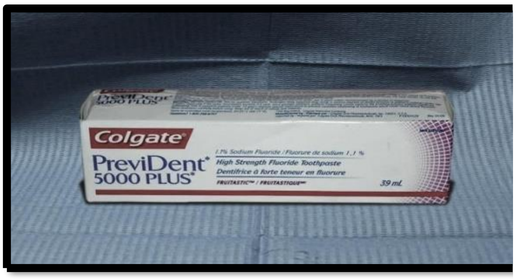

Figure 1: DuraShield CV Clear Varnish **Figure2: Colgate Previ-Dent Toothpaste**

Figure 3: MI (CPP-ACP)Varnish **Figure 4: MI (CPP-ACP) Paste Plus**

Figure 5: Clinpro (TCP) White Varnish

Figure 6: Clinpro (TCP) 5000 paste

Table (1): Materials' specification, composition, lot number and manufacturers

Product	***Composition***	***Lot #***	***Company***
DuraShield® CV 5%Sodium Fluoride Clear Varnish	5% (w/w) sodium fluoride in an alcohol solution of synthetic resin and rosin. Each unit-dose package contains an average of 20 mg of sodium fluoride (NaF), equivalent to 9 mg fluoride ion.	140736	Sultan healthcare, U.S.A.
Colgate™ PreviDent®5000 plus Toothpaste 1.1% Sodium Fluoride	Active Ingredient Sodium fluoride 1.1% (w/w). Inactive Ingredients water, sorbitol, hydrated silica, PEG12, tetrapotassium pyrophosphate, sodium lauryl sulfate, flavor, xanthan gum, sodium benzoate, sodium saccharin, FD&C blue.	41210sc11a	Colgate Oral, U.S.A.
MI Varnish™ 5% Sodium Fluoride Varnish CPP-ACP	5% sodium fluoride varnish with RECALDENT™ (CPP-ACP).ethyl alcohol 25-50%, Rosin, hydrogenated , ethoxyethanol, polyvinyl acetate	1312102	GC corporation, Japan

MI Paste Plus™ 0.2% Sodium Fluoride with CPP-ACP	0.2% w/w fluoride ,Pure water, Glycerol, CPP-ACP, D-sorbitol, CMC- Na, Propylene glycol, Silicon dioxide, Titanium dioxide, Xylitol, Phosphoric acid	141114t	GC corporation, Japan
Clinpro™ 5% Sodium Fluoride White Varnish with TCP	viscous fluid that contains 5% w/w sodium fluoride(22900 ppm F), alcohol ,water, Rosin, Flavors	N 588850	3M ESPE U.S.A
Clinpro™ 5000 1.1% Sodium Fluoride paste with TCP	Paste contains1.1% w/w Sodium Fluoride(5000 ppm F). tri-calcium phosphate.	40072	3M ESPE U.S.A

2. Methods:

2.1. Selection of specimens:

A total number of 108 specimen obtain from 54 of sound freshly extracted human molar teeth were used in this study. The teeth were examined by magnifying loupes[1] to ensure that they were free from caries, cracks, fractures, restorations or any pathological abnormalities. The teeth were then cleaned and washed thoroughly under running water to remove blood followed by gentle scaling[2] to remove any plaque or calculus or any attached periodontal tissue, then stored in a physiological saline solution containing 10%Sodium azide until used.

2.2. Grouping of specimen:

The Specimens were randomly divided into three main groups according to type of varnish used with 36 specimens each, where (A_1) demineralized enamel treated with *Fluoride remineralizing varnish*, (A_2) demineralized enamel treated with *CPP-ACP remineralizing varnish* and (A_3) demineralized enamel treated with *TCP remineralizing varnish*. Each treated group were further divided into four subgroups (B) according to the applied remineralizing paste with 9 specimen each, where (B_0) represent *no paste application*, (B_1) represent *fluoride paste application*, (B_2) represent *CPP-ACP paste application* and (B_3) represent *TCP paste application* ***(Table 2&3)***.

➢ **Table (2): variable of study**

Variable	symbol	Refer to
Remineralizing Varnish (A)	A1	Fluoride
	A2	CPP-ACP
	A3	TCP
Paste Application (B)	B0	No paste
	B1	Fluoride
	B2	CPP-ACP
	B3	TCP

➢ **Table (3): interaction of variable**

Varnish / Paste	A1	A2	A3	Total
B0	A1 B0	A2 B0	A3 B0	27
B1	A1 B1	A2 B1	A3 B1	27
B2	A1 B2	A2 B2	A3 B2	27
B3	A1 B3	A2 B3	A3 B3	27
Total	36	36	36	108

(N=9)

2.3. Preparation of specimen :

Teeth were sectioned into bucco-lingual direction into two halves (Mesial and distal halves) using gold diamond cutting disc with a low speed handpiece and water coolant. Each half of the sectioned tooth was embedded in acrylic resin[3] block using plastic mold, then initial surface roughness and micro- hardness was measured for all specimens as baseline ***(Fig.7, 8)***.

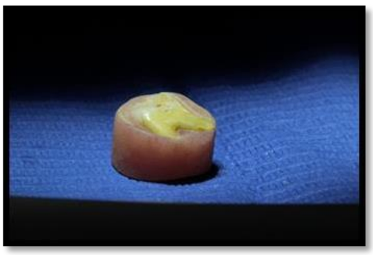

Figure 7: low speed contra **Figure 8: specimen impeded in**
acrylic resin with gold diamond disc

2.4. <u>Preparation of white spot lesion:</u>

The specimen were soaked in the previously mentioned demineralizing solution in a proportion of 2 ml solution/mm^2 of exposed enamel for 72 hours to induce caries like lesion ***(Lata, et al., 2010)***. After that the teeth were removed from the demineralizing solution, washed with water and put on dried absorbent paper, then again both surface roughness and micro-hardness of demineralized specimens were measured.

2.5. <u>Application of remineralizing agent:</u>

All remineralizing agents were applied according to manufacture instructions.

2.5.a. <u>Application of varnish:</u>

All the remineralizing varnish were applied by micro brush then left undisturbed and allowed to set for 4 hours according to manufacturer instruction, which mentioned that patient should be advised not to eat or drink for 4 hours till setting of varnish, after that the toothpaste was applied then subjected to pH cycle protocol ***(Fig.9)***.

25.b. <u>Application of remineralizing paste</u>

To standardize the volume of applied material, each paste was inserted into an insulin syringe[4] then 0.2 ml (20 units) of all agents was used to cover the surface of the specimens. Then the paste was rubbed on specimen surface by micro brush and the application time was adjusted to be 5 minutes as recommended by manufacturer. After that the excess paste was removed and washing specimens in distilled water before application of pH cycling protocol ***(Fig.10)***.

Figure 9: Varnish application

Figure 10: paste application

3D image of the surface profile of the specimens was created. Five 3D images were collected for each specimen, in the central area and in the sides at area of 10 µm × 10 µm, then WSxM software was used to calculate average of heights

(Ra) expressed in µm ***(Fig. 12&13)***.

Figure 12: USB Digital microscope connected to computer

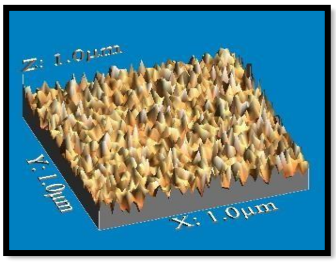

Figure 13: 3D image of the specimen surface profile of the specimens

Results :

The result of current study show that the highest microhardness recovery record by TCP groups either varnish or paste followed by CPP-ACP and finally the least recovery recorded by fluoride either paste or varnish. This result was in agreement with (Karlinsey, et al., 2010a) who clarified that remineralization benefits of TCP is achieved by its ability to penetrate deep into the subsurface lesion. (Elkassas and Arafa, 2014) also reported that fTCP is produced by functionalizing β-TCP with silica, which provides linking opportunities with hard tissue defects under acidic conditions. It can permeate throughout enamel without attacking the inter-prismatic organic material, which may encourage greater calcium, phosphate, and fluoride uptake in demineralized lesions

Discussion :

The goal of modern dentistry is to manage non-cavitated carious lesions non-invasively in an attempt to prevent further disease progression and preserve integrity of healthy tooth substrate **(Goswami, et al., 2012)**. Non-invasive management of white spot carious lesions takes several strategies; among these is healing the demineralized lesions by using remineralizing agents like pastes and resinous varnishes **(Elkassas and Arafa, 2014)**.

Nevertheless, the substance may also exert undesirable effects such as fluorosis and toxicity in high doses. Therefore, efforts to find effective anti- caries compounds with minimal side effects have been intensified (**Tavassoli- Hojjati, et al., 2012**). In consequence this study was conducted to assess the effect of different remineralizing agents with different application technique on enamel white spot lesion by micro- hardness and surface roughness test.

Three different remineralizing agents with different form either paste or vanish were used in this study; the first one is the fluoride remineralizing paste and varnish. Fluoride is believed to prevent dental caries through several mechanisms inducing reduction of acid production by microorganisms, inhibition of intracellular and extracellular enzymes, and replacement of hydroxide ions in hydroxyapatite with fluoride ions (resulting in acid-resistant fluorapatite crystals).

Application compared to fluoride gel. Varnish fluoride hardens after exposure to saliva; consequently, sticking to the teeth result in increased contact time of fluoride with the teeth. Also the high fluoride concentration help in prevention of tooth decay **(Tavassoli-Hojjati, et al., 2012)**.

Furthermore TCP toothpaste (*Clinpro™ 5000)* contains 1.1% (5000 ppm) sodium fluoride was also used in this study. This 1.1% sodium fluoride (NaF) silica-containing paste contains an innovative functionalized tricalcium phosphate (fTCP) ingredient that, able to boost remineralization performance Relative to fluoride-only systems **(Karlinsey et al., 2009)**. The fTCP technology protect bioavailable calcium with a fluoride-repelling surfactant (sodium lauryl sulfate) and as a result, can be readily combined in an aqueous dentifrice formulation with NaF **(Karlinsey, et al., 2010)**. This Anti-Cavity Toothpaste was developed specifically for patients who need the benefits of higher concentration fluoride tooth paste **(Karlinsey et al., 2013)**. In this study, every effort was exerted to standardize the methodology and simulate the clinical conditions. Intact human molars were selected in this study to standardize anatomic variations and to overcome heterogeneous nature of tooth structure.

Teeth were sectioned into bucco-lingual direction into two halves (Mesial and distal halves) for testing whole surface of enamel due to proximal surface in premolars and molars morphology near flat than buccal and lingual surface so its suitable for microhardness test and without flattening due to our main concept in this study not to harm normal structure of enamel for simulation of real life situation as possible **(Heymann, et al., 2014)**.

Artificial carious lesions were obtained by immersion of the specimens in demineralizing solution for 72 hours to create a subsurface demineralization area of approximately 150 microns width with an intact surface simulating an early enamel lesion **(Ekambaram, et al., 2011)**. The 50% concentration of both calcium and phosphates, together with fluoride in order to prevented surface demineralization by forming fluorapatite at the surface, which simulated the naturally occurring early enamel lesions that characterized by subsurface demineralization with intact surface layer **(Lata, et al., 2010).**

The in-vitro pH cycling models are still broadly used because they mimic the dynamics of mineral loss and gain involved in caries process **(Buzalaf, et al., 2010)**. In the current study, the pH-cycling regimen was applied that consist

In this study, both microhardness and surface roughness of each specimen were measured three times; firstly sound enamel as the baseline, and after induction of white spot lesion (demineralization) and finally after application of tested materials under pH cycling regime; this method allows repeated measurements of the same specimen over a given period of time thus reducing the experimental variation **(Mohd Said, et al., 2016).** ***References***:

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