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THE EFFECT OF CARIES INFILTRATION TECHNIQUE (ICONS) ON SURFACE ROUGHNESS AND MICROHARDNESS OF ENAMEL WHITE SPOT LESIONS

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ABSTRACT

This research was designed to evaluate the effect of resin infiltration material (Icon) and remineralizing agents casein phosphopeptide- amorphous calcium phosphate (CPP-ACP) and Nano hydroxyl apatite (n-HA) on surface roughness and microhardness of enamel white spot lesion**.** A total number of 120 sound non-carious human anterior teeth were collected. The labial surfaces of all specimens are treated by 37% phosphoric acid for one minute to create artificial enamel white spot lesion. The teeth were divided into two main groups (60 teeth each); Group H: for assessment of enamel microhardness and Group R: for assessment of surface roughness. Each main group was further divided into four equal subgroups (15 teeth each) according to the treatment applied as follows: Subgroup (C): control, Subgroup (P): CPP-ACP, Subgroup (I): Icon, Subgroup (N): Naohydroxyapatite. Each subgroup was further subdivided into three equal divisions (5 teeth each) according to the storage period in artificial saliva; one week (1W), four weeks (4W) and eight weeks (8W). For assessment of microhardness; the enamel white spot lesion specimens in the four different subgroups were examined three times (after one week, four weeks and eight weeks from the first time of treatment application) by Digital Display Vickers Micro-hardness Tester. The results of microhardness revealed that the subgroup (N) group was provided the highest hardness mean value, followed by subgroup (I), then subgroup (P) while subgroup (C) showed the lowest microhardness mean value. For assessment of surface roughness; the enamel white spot lesion specimens in the three different subgroups were examined three times (after one week, four weeks and eight weeks from the first time of treatment application) by non-contact profilometer. The results of surface roughness revealed that the subgroup (P) was provided the highest roughness mean value, followed by subgroup (C) group while subgroup (I) showed the lowest surface roughness mean value.

INTRODUCTION

The clinical signs of enamel caries are called 'white spot' lesions due to the difference of the refractive indices of adjacent sound enamel and air or electrolytes contained in the porosities of the lesion. Initial enamel caries should be detected early to promote the remineralization before cavitation occurs as well as to stop the progression of the lesions by conservative treatments (1). A number of noninvasive or micro invasive procedures have been introduced to both arrest caries progression or further remineralize the lesion as resin infiltration (Icon) and use topical remineralizig gents as casein phosphopeptide- amorphous calcium phosphate (CPP-ACP) and Nano hydroxyl apatite (n-HA) (2). Caries infiltration technique aims to arrest the progression of the initial enamel caries by filling up the subsurface porosities of the lesions with a low viscosity, light-curing material, and so-called resin infiltrants (3). casein phosphopeptide- amorphous calcium phosphate (CPP-ACP) is milk-derived compounds, in which amorphous calcium phosphate is stabilized. CPP-ACP has been shown to reduce demineralization and promote remineralization of initial carious lesions by elevating the level of free calcium and phosphate ions at the tooth surfaces (4). Hydroxyapa-

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tite is one of the most biocompatible and bioactive materials. Nano-sized particles have similarity to the apatite crystal of tooth enamel in morphology and crystal structure. Micro hardness testing has been shown to be a relatively simple and reasonably reliable method for the provision of indirect information on mineral content changes in dental hard tissues (5). Surface roughness (Ra) refers to the finer irregularities of the surface texture that usually result from the action of the production process or material condition and is measured in micrometers (μm). Aziznezhad et al. (6) compared the effect of resin infiltrant, fluoride varnish, and nano-hydroxy apatite paste on the surface hardness and adhesion of Streptococcus mutans as noninvasive treatments for initial enamel lesions. They concluded that the resin infiltrant increased the tooth surface hardness as the intact enamel and fluoride varnish had the highest reduction level for bacterial adhesion. Nano P paste had an effect between the two materials, both in increasing surface hardness and reducing bacterial adhesion. Pintanon et al. (7) determined the color improvement and surface hardness recovery of artificial white spot lesions after treatment with Icon and CPP-ACP. They concluded that caries infiltration with resin material is shown to immediately improve the surface hardness and esthetic appearance of artificial white spot lesions in vitro. CPP-ACP remineralizing paste is unsuccessful in the enhancement of physical and mechanical characteristics of initial enamel caries during 8 weeks of demineralizing-remineralizing regimen. Mohammed. et al. (8) evaluated the micro hardness and surface roughness of the artificial demineralized enamel treated with resin infiltration system (Icon) and fluoride gel. They concluded that Icon enhancing the micro hardness and decrease surface roughness of the demineralized enamel more than NaF gel. Haghgoo. et al. (9) evaluated the effect of Nano- hydroxyapatite (n-HA) on erosive lesions of the enamel of permanent teeth following exposure to soft beer in vitro. They concluded that n-HA has the potential to remineralize erosive enamel lesions caused by exposure to soft beer. The aim of the study is directed to evaluate the effect of (Icon, casein phosphopeptide- amorphous calcium phosphate (CPP-ACP) and Nano hydroxyl apatite (n-HA) on Micro hardness Surface roughness of enamel white spot lesion.

SUBJECTS AND METHODS

Materials:

1. Resin infiltrant material (Icon).
2. Casein phosphopeptide - Amorphous calcium phosphate (CPP-ACP) in the form of tooth mousse paste as a remineralizing agent.
3. Nano hydroxyapatite (n-HA) in the form of solution
4. Demineralizing agent (37%phosphoric acid).
5. Artificial saliva. Artificial saliva and (n-HA) solution were prepared in Faculty of Pharmacy, Al-Azhar University.

Methods:

A total number of 120 sound non-carious human anterior teeth were collected from oral surgery clinic, free of cracks and any developmental defects, were used in this study. The teeth were washed under running water to remove blood and debris, scaled to remove calculus and remnants of periodontal tissue and polished with fine pumice and soft rubber cups rotating at low speed under water coolant. Teeth were stored in distilled water at room temperature until use. The distilled water was changed daily. The teeth were divided into two main groups (60 teeth each) according to the way of assessment; Group H: for assessment of enamel microhardness and Group R: for assessment of surface roughness. Each main group was further divided into four equal subgroups (15 teeth each) according to the treatment applied as follows: Subgroup (C): control, Subgroup (P): CPP-ACP, Subgroup (I): Icon, Subgroup (N): Nao-hydroxyapatite. Each subgroup was further subdivided into three equal divisions (5 teeth each) according to the storage period in artificial saliva; one week (1W), four weeks (4W) and eight weeks (8W). The crowns of all teeth were separated from the roots by a diamond-coated band saw under continuous water cooling. A specially fabricated circle plastic mold of internal diameter 25mm and 10mm in height was fabricated. The labial surfaces of all specimens were divided into three thirds horizontally then applying phosphoric acid $H_3 PO_4$ 37% to the middle third for one minute to create artificial white spot lesions.

Surface treatments:

In subgroup (A) 15 specimens were immersed in artificial saliva without surface treatment and the artificial saliva changed daily. In subgroup (I) Icons was applied on labial surface of specimens according to manufacture instructors as the followings, *Icon-Etch* was applied and allowed to sit for 2min. etchant was rinsed off with water for at least 30s, and specimens were air dried. Icon-Dry was applied to the lesion site and left for 30s, followed by air drying. Icon-Infiltrant was applied to the etched surfaces and allowed to sit for 3min, then light-cured for 40 s using Monitex BlueLuxcer M-835. Icon-Infiltrant was repeated and allowed to sit for 1min. Specimens were polished with aluminum oxide abrasive papers for20s. In subgroup (P) tooth mousse a CPP–ACP- containing paste were applied to the labial surfaces of specimens then brushed manually by a soft toothbrush and with minimum pressure; brushing procedures were carried out three times daily for 3 minutes then immersed in artificial saliva. **In Subgroup (N)** The specimens were immersed in n-HA solution for 3min then immersed in artificial saliva and these applications were repeated daily*:*

Measurements of specimens

For microhardness assessment: Surface microhardness of the specimens was determined using Digital Display Vickers Micro-hardness Tester with a Vickers diamond indenter and a 20X objective lens. A load of 200g was applied to the surface of the specimens for 20 seconds. Three indentations, which were equally placed over a circle and not closer than 0.5 mm to the adjacent indentations, were made on the surface of each specimen. The diagonals length of the indentations was measured by built in scaled microscope and Vickers values were converted into micro-hardness values. Micro-hardness calculation; - Micro-hardness was obtained using the following equation: - HV=1.854 P/d2 where, HV is Vickers hardness in Kgf/mm2, P is the load in Kgf and d is the length of the diagonals in mm.

For surface roughness assessment: Enamel surface roughness of each specimen was determined using non-contact profilometer. The optical methods tend to fulfill the need for quantitative characterization of surface topography without contact Specimens were photographed using USB Digital microscope with a built-in camera* connected with an IBM compatible personal computer using a fixed magnification of 90X.The images were recorded with a resolution of 1280 × 1024 pixels per image. Digital microscope images were cropped to 350 x 400 pixels using Microsoft office picture manager to specify/standardize area of roughness measurement. The cropped images were analyzed using WSxM software Within the WSxM software, all limits, sizes, frames and measured parameters are expressed in pixels. Therefore, system calibration was done to convert the pixels into absolute real-world units. Calibration was made by comparing an object of known size (a ruler in this study) with scale generated by the software. Subsequently, a 3D image of the surface profile of the specimens was created. 3D images were collected for each specimen, both in the central area and in the sides at area of 10μm × 10 μm WSxM software was used to calculate average roughness expressed in µm which can be assumed as a reliable indices of surface roughness

RESULTS

Irrespective of storage time, totally it was found that Nano- treated group recorded the highest micro hardness mean values followed by Icon group then CPP-ACP while control group recorded the lowest micro hardness mean value. The difference between treatment groups and control group was statistically significant. The difference between (Icon, CPP-ACP and control) groups was statistically non-significant as shown in table (1).

Regardless to treatment group, totally it was found that eight weeks stored subgroups recorded the highest micro hardness mean values followed by four weeks stored subgroups while one-week stored subgroups recorded the lowest micro hardness mean value. The difference between different storage times was statistically significant. While the difference between (four weeks and one week) stored subgroups were statistically non-significant as shown in table (2).

TABLE (1): Micro hardness results (Mean, ±SD) for all treatment groups.

Variable		*One week*	*four weeks*	*eight weeks*	*ANOVA*
		Mean±SD	Mean±SD	Mean±SD	P value
Treatment groups	*Icon*	308.7987±16.9	303.0333±11.4	288.4002±3.32	0.3907 ns
	CPP-ACP	288.0832±6.2	299.5926±15.3	304.9792±26.5	0.5313 ns
	n-HA	301.4889±*3.1*	309.9664±*20.4*	323.2565±*5.2*	0.1032 ns
Control		275.9195±*10.5*	291.2616±*3.26*	306.2866±*4.1*	0.0027*

ns; non-significant (p>0.05) **; significant (p<0.05)*

TABLE (2) Surface roughness results (Mean±SD) for all treatment groups.

Variable		*One week*	*Four weeks*	*eight weeks*	*ANOVA*
		Mean±SD	*Mean±SD*	*Mean±SD*	*P value*
Treatment groups	*Icon*	0.2555±0.3	0.2564±0.07	0.2574±0.02	0.3740 ns
	CPP/ACP	0.2608±0.4	0.2583±04	0.2576±0.02	0.0831 ns
	n-HA	0.2581±0.1	0.2579±0.0032	0.2550±0.01	0.0297*
Control		0.2584±0.7	0.2573±0.05	0.2560±0.03	0.3857 ns

ns; non-significant (p>0.05) **; significant (p<0.05)*

DISCUSSION

In the present study results table (1) revealed that the Nano treated group provided highest micro hardness mean value followed by Icon treated group and followed by casein treated group while the control group provided the lowest micro hardness mean value (291.155). This result was explained by increase deposition of calcium and phosphate ions from n-HA into demineralized enamel which occluded enamel porosities [10]. Also, Nano-hydroxyapatite is closely resembling the morphology of the original apatite crystals in the dental tissue as hydroxyapatite crystals in the tooth structure are generally in the form of needle-like crystals and this will enable forming of biomimetic apatite coating the enamel surfaces. This result was in agreement with the Amaechi. et al. [11] in 2015, who concluded that Nano-particulate hydroxyapatite may act as a calcium and phosphate reservoir, helping to maintain a topical state of super saturation with time for these ions with respect to tooth minerals.

In the present study results table (1) revealed that the Icon treated group provided the higher micro hardness mean value than casein treated group while the control group provided the lowest micro hardness mean. This result was explained by ability of low-viscosity resin to fill the spaces between the remaining crystals of porous lesions and create a diffusion barrier not only at the surface, but also within the enamel lesion body. Therefore, resin-infiltrated layer able to strengthen the demineralized enamel structure and prevent further wear and cavitation[12]. Moreover, some researchers reported that the penetration depth of 15% HCL etching is more than twice that of phosphoric acid, enabling penetration into the deepest part of the lesion and eliminating the decalcified areas [13]. The surface is then dehydrated with 99% ethanol (Icon dry) improve the efficacy of penetration of the hydrophobic infiltrate (TEGDMA) to get a well-defined, resin-infiltrated layer [14, 15]. The result of this study was in agreement with Arslan. et al. [12] in 2015, who showed an increase in micro hardness and a decrease in roughness of demineralized enamel surfaces after application resin infiltration technique which was capable of arresting initial enamel carious lesions. The result of this study was in disagreement with Taher et al in (2012) who concluded that the Icon surface pretreatment sealed the enamel porosities in the infiltrated enamel and but it not improves the surface hardness compared to sound enamel [10].

The results of this study Table 1 revealed that the casein treated group provided higher micro hardness mean value than control groups. This result was explained by ability of ACP to localize at the tooth surface which then buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of super-saturation with respect to the enamel, so depressing demineralization and promoting remineralization of hard tissues [16]. This result was in agreement with Heshmat. et al [17] who concluded that the remineralizing products such as MI Paste Plus (CPP-ACP) and Remin Pro or natural saliva can increase the hardness of enamel surfaces which have been decreased following bleaching agent's application. This result was in disagreement with Pintanon et al [7] who showed that the daily application of CPP-ACP containing paste did not regain the surface hardness of the lesions and the color change could not be significantly observed within 8 weeks of pH cycle.

In the present study results table (1) revealed that the Icon treated group provided the highest micro hardness mean value at one week followed by four weeks while it provided the lowest micro hardness mean value at eight weeks. This can be explained by ability of resin infiltration (Icon) to increase the micro hardness of the demineralized enamel immediately but later on the micro hardness decreased due to water softening of TEGDMA polymer networks and partial dissolution of the remaining mineral in the lesion body that was not completely embedded within the resin matrix or by the resin shrinkage after light curing, which results in leakage [7, 18, 19]. This result was confirmed with Pintanon. et al [7] who concluded that caries infiltration with resin material is shown to immediately improve the surface hardness and esthetic appearance of artificial white spot lesions in vitro. Also found that the Icon provided the highest micro hardness at one day followed by one week while eight weeks provided the lowest enamel micro hardness.

In the present study results table (1) revealed that the (CPP-ACP, n-HA & artificial saliva) treated group provided the highest micro hardness mean value at eight weeks followed by four weeks while it provided the lowest micro hardness mean value at one weeks. This result was explained by increase deposition of calcium and phosphate ions from the surface treatment materials (CPP-ACP, n-HA& artificial saliva) into demineralized enamel by increase time of application. The results of this study were in agreement with Huang. et al [20] and Kim. et al[23] who demonstrated that surface hardness of the demineralized enamel increased with increasing Nano- hydroxyapatite concentration by time, in the present study results (table 2) revealed that the casein treated group provided highest surface roughness mean value followed by control group then Nano treated group while the Icon treated group provided the lowest surface roughness mean value. This result can be explained by the Icon is micro invasive method used to avoid the sacrifice of healthy external intact enamel layer which is important layer of the enamel smooth surface. The result of this study was in agreement with Bakaa. et al [22] who concluded that the resin infiltration decreased surface roughness caused by enamel demineralization. The result of thisstudy was in disagreement with Ina Ulrich. et al [23] who concluded that the resin infiltration does not improve the surface roughness of natural proximal subsurface lesions. Because the micro porosities which were created with hydrochloric acid will not completely filled with the resin infiltration material so the surface roughness will increase. The results of this study (table 2) revealed that the Nanohydroxy apatite treated group provided lower surface roughness mean value than the control group while casein treated group provided the highest surface roughness mean value. This results can be explained by similarity of Nano-sized particles of n-HA to the apatite crystal of tooth enamel in morphology and crystal structure [24]. The result of this study was in agreement with Millcreek. et al [25] who showed that the remineralization therapies with toothpaste containing Nanohydroxy-apatite led to significant increase of Surface micro hardness and decrease surface roughness of artificial enamel white spot lesion.

The results of this study were in disagreement with Ajami. et al [26] who reported that there was no statistically significant reduction in roughness parameters after 10 days of Nanohydroxyapatite serum or n-HA toothpaste application. Nanohydroxyapatite could not restore enamel surfaces to their original condition after orthodontic debonding procedure.

The results of this study Table (2) revealed that the casein treated group provided higher surface roughness mean value than control group. These results can be explained by increase enamel surface roughness by mechanical brushing with (CPP-ACP). Also, the artificial saliva used for storage which contains calcium and phosphate in concentrations similar to those found in human saliva lead to slightly increase in mineral in control group. This result was in agreement with Amaral. et al. [27] who reported that that tooth brushing with dentifrices with high concentration of NaF and CPP-ACPF cream was not able to prevent enamel erosion in simulated oral environment. Significant increase in Surface roughness was observed for all groups. The result of this study was in disagreement with Bakaa. et al.[22] who reported that the remineralization by

(CPP-ACP) procedures decrease surface roughness caused by enamel demineralization.

The results of this study (tables 1and 2) revealed that the treated groups at eight weeks provided the highest micro hardness mean value and lowest surface roughness mean value followed by treated groups at four weeks while the one-week results provided the lowest micro hardness value and highest surface roughness mean value. This result was explained by increase deposition of calcium and phosphate ions from the surface treatment materials (CPP-ACP, n-HA & Artificial saliva) into demineralized enamel by increase the time of application and immediately closure the enamel porosities with resin infiltration [8, 13]. The result of this study was in agreement with Huang. et al. [20] and Kim et al. [21] who demonstrated that surface hardness of the demineralized enamel increased with increasing Nano- hydroxyapatite concentration by time.

The result of this study at one-week storage time revealed that Icon treated group recorded the highest micro hardness mean value and lowest surface roughness mean value followed by Nano-group then CAP/CPP while control group recorded the lowest micro hardness mean value and highest surface roughness mean value (tables 1and 2). This result was explained by immediately ability of Icon to closure porosities of demineralized enamel after resin infiltration curing [8]. This result was confirmed with Pintanon et al [7] who concluded that caries infiltration with resin material is shown to immediately improve the surface hardness and esthetic appearance of artificial white spot lesions in vitro.

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