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EFFECT OF CARIES INFILTRATION TECHNIQUE (ICON) ON THE REMINERALIZATION AND COLOR STABILITY 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 (fluoride, CPP-ACP) on remineralization and color stability of enamel white spot lesion**.** A total number of 120 sound non-carious human anterior teeth were collected. The teeth were divided into two main groups (60 teeth each); Group R: for assessment of enamel remineralization and Group S: for assessment of color stability. Each main group was further divided into four equal subgroups (15 teeth each) according to the treatment applied as follows: Subgroup (A): control, Subgroup (P): CPP-ACP, Subgroup (I): ICON Subgroup (F): Fluoride. Each subgroup was further subdivided into three equal division (5 teeth each) according to the storage period in artificial saliva; one week (1W), four weeks (4W) and six weeks (6W). For assessment of remineralization; the enamel white spot lesion specimens in the four different subgroups were examined three times (after one week, four weeks and six weeks from the first time of treatment application) by (SEM/EDX). The results of remineralization revealed that the subgroup (P) group was provided the highest ca/p ratio mean value, followed by subgroup (F), then subgroup (I) while subgroup (A) showed the lowest ca/p ratio mean value. For assessment of color stability; the enamel white spot lesion specimens in the three different subgroups were examined three times (after one week, four weeks and six weeks from the first time of treatment application) by a portable Reflective spectrophotometer. The results of color stability revealed that the subgroup (F) was provided the highest color variation mean value, followed by subgroup (P) group while subgroup (I) showed the lowest color variation mean value.

INTRODUCTION

White spot lesions are clinically visible signs of demineralization of tooth structure. The caries process, in general, involves loss of minerals from the tooth due to metabolic events in biofilms or bacterial plaque. This loss leaves porosities in the enamel which change the optical properties giving the opaque, white appearance [1, 2]. Resin infiltration technique (Icons) is a novel technology providing an intermediary treatment option between prevention and restorative therapy. The principal of resin infiltration is to perfuse the porous enamel with resin by capillary action. This aims to arrest lesion progression by occluding the microporosities that provide diffusion pathways for the acids and dissolved materials [3]. Casein phosphopeptide - Amorphous calcium phosphate (CPP-ACP) is considered as anticariogenic agent used in the treatment of dental caries and other hypo mineralized conditions. CPP-ACP are a safe and novel carrier for calcium, phosphate and hydroxide (fluoride) ions to promote enamel remineralization with application in oral care products, dental professional products and foodstuffs[4]. Fluoride from topical sources speeds up the remineralization process by incorporating into the surface as fluorapatite and attracting calcium and phosphate ions. This new fluorapatite surface has a much lower solubility than hydroxyapatite and can resist further demineralization [5].

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Abdullah et al. [6], evaluated the efficacy of minimally invasive methods for the treatment of white-spot lesions involving fluorides, CPP-ACP (casein phosphopeptide-stabilized amorphous calcium phosphate) and resin infiltration vs a placebo or control. They concluded that minimally invasive treatment modalities of white spot lesions produced significant improvement in the appearance and regression of white spot lesions following treatment when compared to a control or placebo. Doméjean et al. [7] evaluated the in vivo scientific evidence of the ability of resin infiltration (RI) to arrest non-cavitated caries lesions. They concluded that RI appeared to be an effective method to arrest the progression of non-cavitated caries lesions. Additional, long-term studies are required. Gianmaria et al. [8], conducted an in vivo study based on that casein phosphopeptide-amorphous calcium phosphate nanocomplexes (CPP-ACP) exhibit anticariogenic potential in laboratory, animal and human experiments. They found that, based on scanning electron microscope (SEM) analysis, a diffuse and homogeneous mineral coating, reducing the surface alterations only in the demineralized specimens treated with synthetic CPPs into the mouth. They concluded that CPPs are able to promote remineralization of early enamel lesions.

Oliveira et al. [9], compared the remineralization effect on white spot lesions of casein phosphopeptide-amorphous calcium phosphate crème, or CPP-ACP, 1.1% NaF dentifrice containing 5000ppm of fluoride, or CPP-ACP crème with 900ppm of fluoride with that of a control. The 1.1% NaF dentifrice demonstrated overall greater remineralization ability than 10% CPP-ACP crème. However, the 1.1% NaF dentifrice was only as effective as the Control to reduce fluorescence loss. Amely et al., evaluated the camouflage effects by concealment of Post-orthodontic WSLs to sound adjacent enamel (SAE) achieved over 12 months with resin infiltration. They concluded that as color and lightness characteristics of the Icon infiltrant as well as the esthetic camouflage effects achieved by WSL infiltration were not altered significantly or clinically relevant after 12 months, the method of resin infiltration can be recommended for an enduring esthetic improvement of Postorthodontic WSL [10].

Singh et al. [11], evaluated freshly bleached enamel surface of extracted human teeth with CPP-ACP treatment. They found that Surface treatment with CPP-ACP on freshly beached enamel surface, significantly reduced the stain absorption. They concluded that CPP-ACP reduce stain absorption after tooth bleaching. Raha et al. [12], evaluated the effect of two fluoride varnishes on color stability of three resin-based restorative materials. They concluded that color changes following the application of fluoride varnishes were found to be clinically acceptable in all groups. The aim of this study is directed to evaluate the effect of Icon, fluoride, and CPP-APP on surface remineralization and color stability of enamel white spot lesions at different storage times.

SUBJECTS AND METHODS

Materials:

1-Resin infiltrant material (ICONS).

2-Casein phosphopeptide - Amorphous calcium phosphate (CPP-ACP) in the form of tooth mousse paste as a remineralizing agent.

3-Fluoride in the form of sodium fluoride (NAF).

4-Demineralizing agent (37%phosphoric acid).

5-Artificial saliva solution was 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 R: for assessment of enamel remineralization and Group S: for assessment of color stability. Each main group was further divided into four equal subgroups (15 teeth each) according to the treatment applied as follows: Subgroup (A): control, Subgroup (P): CPP-ACP, Subgroup (I): ICON Subgroup (F): Fluoride. Each subgroup was further subdivided into three equal division (5 teeth each) according to the storage period in artificial saliva; one week (1W), four weeks (4W) and six weeks (6W). 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 10mm and 20mm in height was fabricated. The labial surfaces of all specimens were divided into three thirds horizontally then applying phosphoric acid H_3PO_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 application of any remineralizing agent 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 another time 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 middle third of 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 (F):** Topex Neutral PH containing sodium fluoride were applied to the middle third of the surfaces then brushed with for 3 minutes followed by PH cycling then immersed in artificial saliva.

Measurements of specimens

For remineralization: Mineral content assessment (especially calcium and phosphorus) by Scanning Electron Microscope and Energy Dispersive X-Ray (SEM/EDX) fig 8, SEM operated at 10KV & equipped with a detector and XP3 pulse processor (Oxford instruments x-ray microanalysis). The specimen was subjected to the electron beam and the take-off angle was 35°. EDX spectrum images were acquired with an EMiSPEC vision integrated acquisition system. Data was recorded within an average of 6 minutes for each specimen. All specimens were then immersed in the demineralizing agent for 72 hours; the solution was changed daily. After days of immersion the superficial dentinal surfaces of specimens were washed with deionized water to remove the excess of acids then subjected to surface analysis by (SEM/EDX).

For color stability: The specimens' colors were measured using a portable Reflective spectrophotometer (X-Rite, model RM200QC, Neu-Isenburg, Germany) figure 8. The aperture size was set to 4 mm and the specimens were exactly aligned with the device. A white background was selected and measurements were made according to the CIE L*a*b* color space relative to the CIE standard illuminant D65. The color changes (ΔE) of the specimens were evaluated.

RESULTS

With Icon group; it was found that six weeks stored subgroups recorded the highest ca/p ratio mean values followed by four weeks stored subgroups while one-week stored subgroups recorded the lowest ca/p ratio mean value. The difference between different storage times was statistically non-significant as shown in table. With CAP/CPP group; it was found that six-week stored subgroups recorded the highest ca/p ratio mean values followed by four weeks stored subgroups while one-week stored subgroups recorded the lowest ca/p ratio mean value. The difference between different storage times was statistically non-significant. With fluoride group; it was found that six-week stored subgroups recorded the highest ca/p ratio mean values followed by four weeks stored subgroups while one-week stored subgroups recorded the lowest ca/p ratio mean value. The difference between different storage times was statistically non-significant as shown in table (1).

With Icon group; it was found that six weeks stored subgroups recorded the highest color variation mean values followed by four weeks stored subgroups while one-week stored subgroups recorded the lowest color variation mean value. The difference between different storage times was statistically non-significant. With CAP/CPP group; it was found that one-week stored subgroups recorded the highest color variation mean values followed by four weeks stored subgroups while six weeks stored subgroups recorded the lowest color variation mean value. The difference between different storage times was statistically non-significant. With fluoride group; it was found that one-week stored subgroups recorded the highest color variation mean values followed by four weeks stored subgroups while six-week stored subgroups recorded the lowest color variation mean value. The difference between different storage times was statistically significant. Also, the difference between (one week and four weeks) stored subgroups was statistically non-significant as shown in tables 1.

Regardless to treatment group, totally it was found that six weeks stored subgroups recorded the highest ca/p ratio mean values followed by four weeks stored subgroups while one-week stored sub-

TABLE (1) Comparison between the four studied groups according to Ca/p ratio and ΔE.

	Treatment groups			Control	p value
	Icon	**CAP/CPP**	**Fluoride**		
Ca/p ratio	2.26 ± 0.17	2.91 ± 0.27	2.85 ± 0.37	2.24 ± 0.07	0.013*
One week	2.2±0.34	2.7±0.35	2.5±0.24	2.1±0.07	-
4 weeks	2.2+2.52	2.8±0.70	2.7±0.77	2.2±0.05	-
6 weeks	2.4±0.07	3.1±0.83	2.9±0.58	2.3±0.16	-
p	0.140	0.09	0.111	0.113	
ΔE	8.25±2.15	9.08±2.50	9.23±2.86	-	0.677
One week	5.28 ± 3.50	11.26 ± 7.53	12.55 ± 5.39	-	-
4 weeks	6.88 ± 2.86	9.98 ± 3.91	11.50 ± 5.21	-	-
6 weeks	7.83 ± 3.25	7.07 ± 1.80	9.89 ± 3.04	-	-
p	0.140	0.09	0.038*	-	

Same letter indicating non-significant (p>0.05) ns; non-significant (p>0.05)

**; significant (p<0.05) Significant minimum difference*

TABLE (2) Comparison of Ca/p ratio and total color variation mean values as function of storage time

Storage time	One week	4 weeks	6 weeks	p value
Ca/p ratio	$2.57^{a} \pm 0.27$	$2.68^{a} \pm 0.65$	$2.76^{a} \pm 0.62$	0.661
Total color variation	$9.69^{a} \pm 6.23$	$9.45^{a} \pm 4.29$	$8.26^{a} \pm 2.85$	0.549

Same letter indicating non-significant (p>0.05) ns; non-significant (p>0.05)

**; significant (p<0.05) Significant minimum difference*

groups recorded the lowest ca/p ratio mean value. The difference between different storage times was statistically non-significant. Regardless to treatment group, totally it was found that one-week stored subgroups recorded the highest ca/p ratio mean values followed by four weeks stored subgroups while six weeks stored subgroups recorded the lowest ca/p ratio mean value. The difference between different storage times was statistically non-significant as shown in table (2).

DISCUSSION

In the present study the Casein treated groups showed highest remineralization potential with highest ca/p ratio followed by fluoride treated group followed by Icon treated group which showed results superior to control groups. It has been attributed to the ability of CPP-ACP to localize ACP at the tooth surface. CPP-ACP maintains super saturation of calcium and phosphate ions thus modulating the bioavailability of calcium phosphate levels and finally leading to an increase in remineralization [13] Moreover, this result was in agreement with Prestes et al. [14], who suggested that chewing gum containing CPP–ACP is able to enhance the mineral precipitation of eroded enamel. Also, in this study results the effect of fluoride treated group in remineralization of WSLs ca/p ratio was higher than ICON treated group while the control group showed the lowest remineralization with ca/p ratio. This result was explained by the unique mechanism of fluoride remineralization. Fluoride speeds up the remineralization process by incorporating into the surface as fluorapatite and attracting calcium and phosphate ions. This new fluorapatite surface has a much lower solubility than hydroxyapatite and can resist further demineralization and increase remineralization [15].

This result was in agreement with Baka et al., in who concluded that CPP-ACP and fluoride has better remineralization potential than Icon. Showed a decreased remineralization potential when compared to CPP-ACP and fluoride [16]. This result was in disagreement with Zeynep and Sevil in who assessed the remineralization effects of ozone, sodium fluoride, resin infiltration and CPP-ACP on artificially demineralized lesions created in enamel. They concluded that CCP-ACP and resin infiltration applications are more effective on the remineralization of early enamel lesions in comparison to NaF varnish and ozone applications [17]. This may be due to different methodology and materials. Also, the results of this study revealed that the Icon treated group provided higher remineralization potential with ca/p ratio than control groups. This was attributed to the presence of pseudo intact layer formed by obturation of microporosities of the demineralized enamel surface by low viscous resin and possibly by occluding the underlying pores in the carious lesion.

This is in agreement with Kielbassa et al., who hypothesized that, the limited remineralization that is shown by Icon is mainly due to incubation in artificial saliva after the application of remineraliz-

ing agent [18]. In the present study results the ICON treated group provided highest color improvement with lowest color variation followed by casein treated group while fluoride treated group showed the lowest color improvement with highest color variation mean value. This result was explained by the enamel surface and most of the carious lesion bodies were filled with infiltration resin matrix, resulting in nearly the same light reflectivity and performance for color measurement. The results of the present study indicated that non-invasive infiltration technology can change the color of early caries lesions and achieve a good aesthetic effect by masking the chalky spots of early caries so that it appears close to its normal color [19]. This result was also in agreement with Paris et al. [20], who assessed the masking effect of resin infiltration in artificially induced white spot lesions in bovine teeth using different infiltrate materials. Also, this result was in agreement with Yuan H.et al., [21] who revealed that resin infiltration is more effective than NaF or CPP-ACP in providing esthetic improvement of WSLs. This result was in disagreement with Cohen et al. [22], who concluded that lesions infiltrated with Icon underwent greater color change when compared with remineralized lesions, which may represent an esthetic disadvantage for the first-mentioned treatment. This may be due to different methodology and materials.

Also, in the present study the color improvement of the WSLs was higher in casein treated group with color variation followed by fluoride treated group. This is explained by healing process of CPP-ACP. Nano-crystals of ACP are small enough to access de-mineralized areas underneath already re-mineralized surface zone. Therefore, a deep progression of favorable aesthetic appearance is possible [23]. This result was in agreement with Andersson A et al., [24] who concluded that the visual evaluation suggested an aesthetically more favorable outcome of the amorphous calcium phosphate treatments than fluoride. The results of this study the treated groups at six weeks provided the highest remineralization with the highest ca/p ratio followed by treated groups at four weeks while the one-week results provided the lowest remineralization. These results were explained by increase deposition of calcium and phosphate ions from the surface treatment materials (CPP-ACP, fluoride & Artificial saliva) into demineralized enamel by increase the time of application and immediately closure the enamel porosities with resin infiltration [25, 26].

This result was in agreement with Kielbassa et al. [27], who evaluated the remineralization effects of natural saliva and saliva substitutes on enamel and dentin and explain that, there was a slightly increase in mineral wt.% in control group than the demineralized specimens. This was in agreement with Dina Elkassas et al. [25], who reported that Calcium phosphate based remineralizing agents provide superior remineralization effects and greater resistance to acid softening as compared to artificial saliva. Extended period of time had helped to attain more benefits of remineralization regimens application. Also, this result was in agreement with Reynolds et al. [28], who reported that in an in vitro study on human teeth with artificially created white spot lesions, CPP complexes remineralized the lesions at a rate of 3.9 ± 0.8x10-8 moll hydroxyapatite/m2/s. This result was in agreement with Torres et al., [29] who showed that although the Icon results in a significant reduction in lesion progression under demineralizing conditions, some demineralization can still occur after Icon treatment.

The results of this study revealed that the treated groups at six weeks provided the highest color improvement with the lowest color variation followed by treated groups at four weeks while the one-week results provided the lowest color improvement. These results were explained by increase deposition of calcium and phosphate ions from the surface

treatment materials (CPP-ACP & fluoride) into demineralized enamel by increase the time of application and to the deep penetration of the resin infiltrating leading to the plugging of porosities within the WSLs might be the factor improving the color by having a similar light refraction index [25, 26]. This result was in agreement with Andersson et al. [30], who compared two treatment protocols for post-orthodontic white spot lesions: A) daily applications of a dental cream containing CPP-ACP for three months followed by a three-month period of daily tooth brushing with a fluoridated dentifrice and B) daily rinse with a 0.05% sodium fluoride mouthwash combined with a fluoridated dentifrice for six months. They concluded that there was a significant improvement in the clinical appearance of the white spot lesion for both groups. This result was in agreement with Matteo et al. [31], who concluded that Icon can fix the initial esthetic problem associated with white spot lesions, but the resin may become more discolored than other materials over time. This result was in disagreement with Paris S et al. [20], who revealed that not only the lesions were masked and significantly had better color match with normal enamel but also, they resisted discoloration following treatment better than the normal enamel.

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