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Original Research Article

Evaluation of the Human Enamel Surface Morphology after Tooth Bleaching Followed By Remineralizing Agents

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ABSTRACT

Background: Over the last two decades there was dramatic growth and impact of tooth whitening worldwide which raised patients' awareness of the appearance of their smile. The exposure of tooth surface to various dental bleaching agents can affect the morphology and roughness of enamel surface. Many treatments to the bleached enamel have been tested such as calcium phosphate and fluoride remineralizing agents.

Objective: The aim of this in vitro study is to evaluate the human enamel surface morphology after tooth bleaching using at home zoom bleaching agent followed by the application of two different protective pastes (ACP gel, MI Paste Plus).

Methods: Specimens were prepared from 20 human premolars free of caries and defects. The bleaching procedures were performed with 22% carbamide peroxide (at home zoom bleaching). For the remineralization treatment, two protective pastes (ACP, MI Paste Plus) were evaluated. Specimens were randomly assigned to 4 groups of 5 specimens each. The effect of the bleaching agent followed by remineralization agents were examined by scanning electron microscopy and DIAGNOdent pen. One-way analysis of variance (ANOVA) test was performed to compare the mean values of investigated groups. Student t – test was used to compare between two groups.

Results: The results of the present study revealed slight to moderate irregularities and pores on the enamel surface after the bleaching procedure with no loss of superficial structure. Application of the protective pastes on the bleached enamel surface showed deposition of a protective layer on the enamel surface. There was a statistical significant difference between the two protective paste groups ($p < 0.05$) regarding the content of minerals with the better remineralization shown in the MI paste plus group.

Conclusion: The use of the protective agents tested can be useful in clinical practice to reduce the adverse effects on enamel surface after bleaching procedures. However, the protective effect of MI Paste Plus product demonstrated a higher efficacy as compared to ACP relief gel product.

Keywords: Bleaching, Enamel, Carbamide peroxide, Remineralization, ACP, MI paste.

INTRODUCTION

Tooth discoloration is defined as "as any change in the color, hue or translucency of a tooth due to any cause". [(1)] The discoloration could be by extrinsic or intrinsic stains. The process of stain formation is not yet well understood. Pellicle-coated enamel is known to have a net negative charge, so allowing selective adhesion of positive ions to the tooth surface. This has been proved to play an important role in the deposition of stains on the outer surface of the tooth. [(2)] Many variable methods are used to treat tooth discoloration one of them is bleaching. American Dental Association defined

bleaching as "the treatment, usually involving an oxidative chemical that alters the light absorbing and/or light reflecting nature of the material /structure thereby increasing its value (whiteness)". [(3)] Over the last two decades, there was dramatic growth and impact of tooth whitening worldwide which raised patients' awareness of the appearance of their smile; [(4)] Bleaching has become one of the most popular esthetic dental treatments. Bleaching is an oxidation reaction, the exact mechanism of bleaching is still unclear. The enamel to be bleached donates electrons to the bleaching agent. [(5)] Ten percent carbamide peroxide breaks down to 7% urea and 3% hydrogen peroxide. The hydrogen peroxide metabolizes into H2Oand free radicals of oxygen. These free radicals have a single electron, which combines with the chromogens to decolorize or solubilize them. [(2)] The tooth bleaching materials currently used are based primarily on either hydrogen peroxide or carbamide peroxide. Whitening can be visually perceived and measured within a few days or weeks. Both materials may change the inherent color of the teeth, but have various considerations for safety and efficacy. The bleaching is suggested to be a relatively safe procedure, although there is continuous adverse effect have been reported on dental hard tissues, soft tissues, and different restorative materials. [(6)] The known toxicity of H_2O_2 is the primary source of a safety concern because the H_2O_2 is capable of creating free radicals so there is persistent harm to the cells along with oxidative reactions are believed to be the main mechanisms responsible for the observed toxicity of H_2O_2. There are two types of adverse effect of bleaching materials: the possibility of systemic side effects if the bleaching gel were to be ingested and local adverse effects on hard and soft tissues due to direct contact of the gel with the tissues. Safety concerns with potential systemic toxicities of H_2O_2 have largely diminished with research works and collection of the data over the last two decades. So, the primary safety issue for tooth bleaching its potential local adverse effects. Primarily tooth sensitivity, gingival irritation as well as potentially adverse effects on enamel and restorations are the commonly known local risks associated with tooth bleaching. [(7)] The enamel is affected by bleaching primarily in three aspects: loss of the minerals, changes in the surface morphology, and alteration of surface microhardness; the in vitro systems are the most conducted studies to detect the enamel changes. [(7)] In 2004 Çobankara F et.al. investigated the effect of home bleaching product on the roughness and surface morphology of human enamel and dentine they found no statistically significant differences in surface roughness between control teeth and teeth that treated by 10-15% CP. [(8)] While in another study by Klarić E et.al2013, they measured the change in microhardness of enamel and dentine by different bleaching materials and they found a significant reduction in surface microhardness of enamel and dentin which will increase by an increase of bleaching agent acidity. [(9)] To overcome this loss of minerals and subsequent micro-hardness by bleaching agent remineralizing solutions are recommended. There are many remineralizing agents such as fluoride, Calcium Phosphate Based Remineralization, Sugar Substitute, and Ozone. [(10)] The ACP technology requires a two-phase delivery system to retain the calcium and phosphorous ions from reacting with each other before use. The current sources of calcium and phosphorous are two salts, calcium sulfate, and dipotassium phosphate. When the two salts are mixed, they quickly form ACP that can precipitate onto the tooth surface. This precipitated ACP can then dissolve into the saliva and can be readily available for tooth remineralization. [(11)] In Eva Klarić et al.2013, They found significant enhancement of surface microhardness with daily application of ACP on bleached enamel. With the ability of ACP to remineralize the demineralized surface by maintaining a high level of minerals in the surrounding environment by

releasing calcium and phosphate the quality of bleached enamel could be improved. [9] MI Paste Plus™ is a sugar-free, water-based cream paste which was introduced to the American market in April 2007. The active ingredient of MI Paste Plus™ is casein Phosphopeptide-amorphous calcium phosphate with 900ppm Fluoride (CPP-ACPF). [12] Heshmat et.al 2013, found significant raise of enamel roughness after use of 37.5% HP gel. The bleached enamel surface was improved after application of MI paste plus which contains CPP-ACPF. [13] In 2015, Poggio et.al. found that the CPP-ACP agent improves the enamel morphology better than fluoridated protective paste. [14] DIAGNOdent is a device that known to detect non-cavitated pit and fissure caries and early smooth surface caries by 655 nm diode laser. Recently DIAGNOdent have been used by several authors to evaluate and determine the mineralization state of the human teeth. [15-17] So, the aim of the present in vitro study was to evaluate the human enamel surface morphology after tooth bleaching using at home zoom bleaching agent followed by the application of two different protective pastes (ACP gel, MI Paste Plus).

MATERIALS AND METHODS

Specimens Preparation:

Specimens were prepared from 20 human premolars free of caries and defects, extracted for periodontal and orthodontic reasons. After the extraction, the teeth were cleansed of soft tissue debris and inspected for cracks, hypoplasia and white spot lesions. They were disinfected in 5.25% sodium hypochlorite solution for 1h and stored in 37^0C buffered saline during the whole experimentation.

Bleaching Procedure and Remineralization:

The bleaching procedures were performed with one of the new at home bleaching material such as at home zoom bleaching agent. For the remineralization treatment, two different protective pastes (ACP, MI Paste Plus) were evaluated. The characteristics, chemical composition and manufacturer of the products are reported in Table I.

Specimens were randomly assigned to 4 groups of 5 specimens each according to the treatment:

group1: intact enamel (control, no treatment will be done);

group 2: enamel +At home zoom bleaching

group 3: enamel + At home zoom bleaching + ACP

group 4: enamel + At home zoom bleaching + MI Paste Plus

The control specimens (group 1) were on storage for the whole experimentation and they were not receiving any treatment. The bleaching treatment with 22% carbamideperoxide (Philips at home zoom bleaching) of groups 2, 3, 4 were performed according to the manufacturer's instructions. at home zoom bleach were applied directly from the mixing tip onto the labial surfaces of the teeth in a 1 to 2mm thick layer. Then at home zoom bleach were wiped off with distilled water washing after 2 hours from its application.

The protective pastes were applied onto the surface of the specimens of groups 3 and 4 without brushing for 15 min and then were wiped off with distilled water washing. In groups 3 and 4the remineralization pastes were applied after the whitening treatment with carbamide peroxide.

Scanning Electron Microscopy:

The specimens were gently air dried, dehydrated with alcohol and then dried at the critical point - a method used to minimize specimen distortion due to drying tensions. Following the desiccating procedure, the specimen was gold sputtered then observed under a SEM, Model Quanta 250 FEG (Field Emission Gun) attached with EDX Unit (Energy Dispersive X-ray Analyses), with accelerating voltage 30 K.V., magnification14x up to 1000000 and resolution for Gun.1n) FEI company, Netherlands. The superficial morphology of enamel was examined and scored as follows: 0, enamel with smooth surface

morphology; 1, enamel with slight irregularities; 2, enamel with moderate irregularities; 3, enamel with accentuated irregularities.

TABLE 1 Materials used in this study

Product	Manufacture	Ingredient	Application	Active agent	%
Zoom	Philips	Propylene Glycol, Glucerin, Water(Aqua), Carbamide Peroxide, Hydrogen Peroxide, Dicetyl Phosphate (AND) Cetearyl Alcohol(AND) Cetetch 10 Phosphate, Silica, Hydroxpropyl Methylcellulose, Potassium Nitrate, Menthe Piperita, Sodium Phosphate, Calcium Nitrate, Calcium Carbonate, Potassium Hydroxide.	At home	Carbamide peroxide	22
ACP		ACP, 5% potassium nitrate, 0.22% sodium fluoride, water, natural peppermint, calcium nitrate, sodium phosphate, sodium saccharine.	In office/at home	Amorphous calcium phosphate	0.22%
MI paste plus	GC	Pure water, Glycerol, CPP-ACP, D-sorbitol, CMC-Na,Propyleneglycol, Silicon dioxide, Titanium dioxide, Xylitol, Phosphoric acid, Sodium fluoride, Flavoring, Sodium saccharin, Ethyl phydroxybenzoate, Propyl phydroxybenzoate, Butyl phydroxybenzoate	In office/at home	CPP-ACP+900ppm F	.2%

Measurements the mineral contents in enamel:

DIAGNOdent was used to measure the amount of mineral loss in the teeth before, after bleaching and after application of remineralizing agent. Score from (0 – 7) indicated normal enamel content and score (12 – 25) indicated initial carious lesion or loss of minerals in the enamel.

Statistical Analysis: The data was tabulated and analyzed using SPSS statistical analysis package version 17. One-way analysis of variance (ANOVA) test was performed to compare the mean values of investigated groups. Student t – test was used to compare between two groups.

RESULTS

Scanning Electron Microscopy Observations

Figure 1(a & b) showed the untreated specimen (group 1) with smooth and regular enamel surface morphology. The bleaching treatment with 22% carbamide peroxide (Philips at home zoom bleaching) (group 2) showed alterations in surface smoothness and different etching patterns in form of slight to moderate irregularities and pores (fig.2 a&b). The application of ACP relief gel on bleached enamel surface in group (3) showed partial remineralization as some evidence of demineralization still observed on the surface in form of pores and irregularities (fig.3 a&b).While the application of MI Paste Plus on bleached enamel surface (group 4) showed better remineralization of the enamel surface with the deposition of a protective layer on the enamel surface (fig.4 a&b).

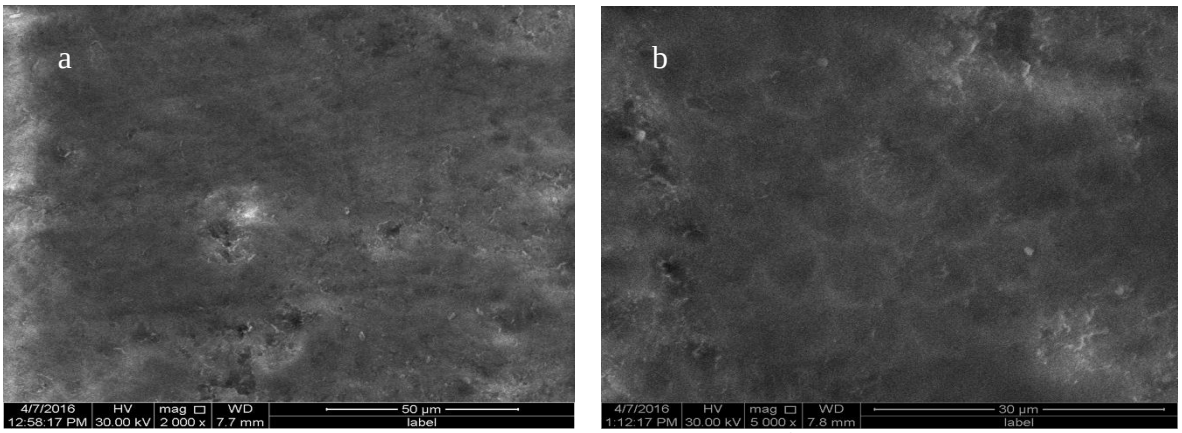

Fig 1: Scanning electron micrograph showing untreated enamel surfaces. (a X2000, b X5000)

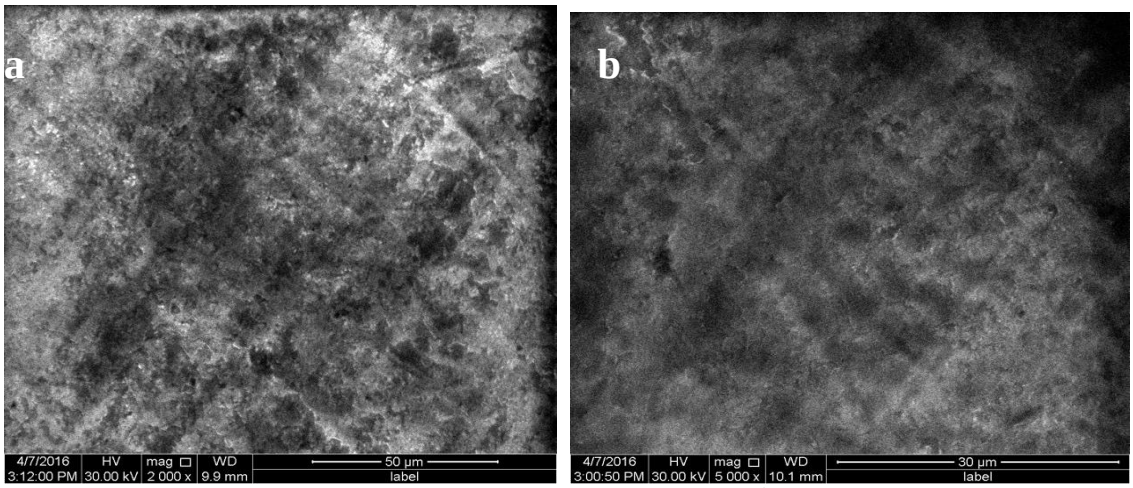

Fig 2: Scanning electron micrograph showing bleached enamel surfaces. (a X2000, b X5000)

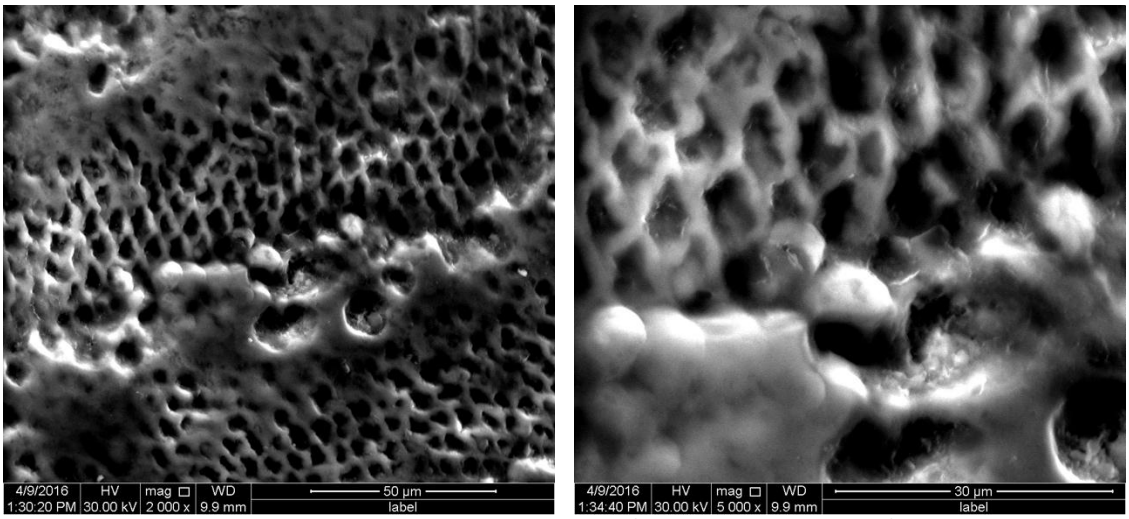

Fig 3: Scanning electron micrograph showing bleached enamel surfaces treated with ACP relief gel. (a X2000, b X5000)

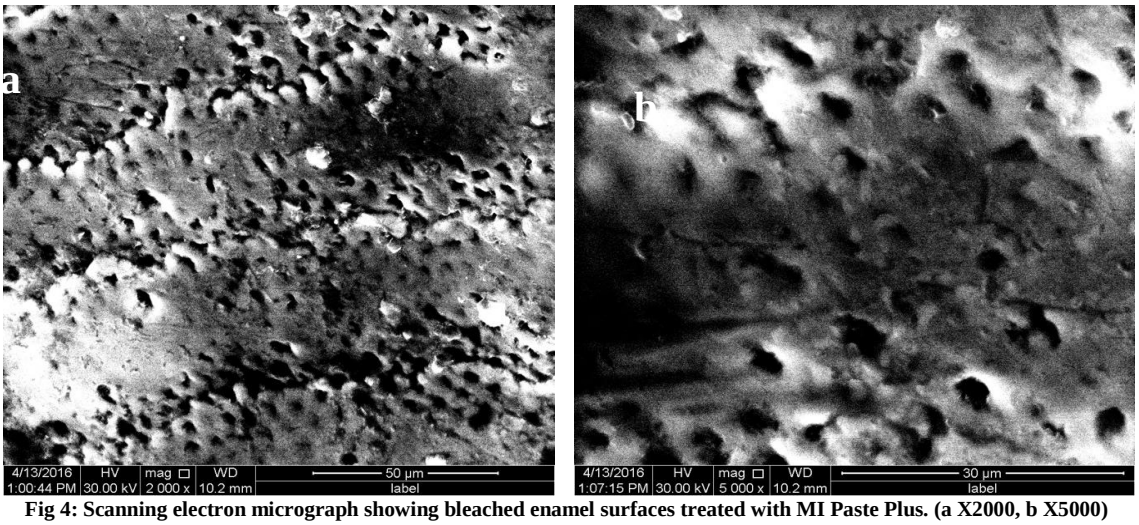

Fig 4: Scanning electron micrograph showing bleached enamel surfaces treated with MI Paste Plus. (a X2000, b X5000)

Measurements of the mineral contents in enamel

Table (2): Mean and standard deviation (SD) of DIAGNOdent measure of the specimens in the four testing groups.

	Bleaching (Mean ±SD)	ACP (Mean ±SD)	MI paste (Mean ±SD)	F (p)
Before bleaching	0.6± 0.527	0.5± 0.634	0.7± 0.531	1.659(0.117)
After Bleaching	7.21± 3.242	8.31± 4.180	7.92± 4.014	1.934(0.09)
After remineralization	6.89± 4.825	1.80 ± 0.421^{AB}	1.30 ±0.465^{AB}	4.457(0.002)*

*Significant at p level <0.05, F (one-way ANOVA test)

A significant difference between the same group after bleaching and after remineralization
B significant difference between the ACP and MI groups after remineralization

Control group DIAGNO dent mean score was (0.5± 0.561) which showed non significant difference between it and the other groups before the bleaching. Other groups were treated with bleaching agents then with remineralizing agents. No significant differences were found between the specimens in the three treated groups before bleaching. Similarly, after application of bleaching agent no significant difference was found between the all specimens in the three groups (p= 0.09). After treatment, ACP and MI paste groups showed lower mean DIAGNO dent scores after remineralization compared with the bleaching group which had no treatment. Significance differences were found (p= 0.002). (table 2)

DISCUSSION

Adverse effects of the bleaching agents on the dental tissues must be considered. The bleaching agents could lead to the dissolution of both organic and inorganic dental matrices by oxide reduction reaction. [18] Several studies evaluated the relation between concentrations of hydrogen peroxide or carbamide peroxide and the decrease in enamel and dentin microhardness and the subsequent morphological changes. [9,14] The results of the present study revealed that intact enamel exposed to at home Zoom bleaching agent (group 2) showed slight to moderate porosities and superficial alterations as compared to the smooth non-treated enamel surface (group 1). These findings are in agreement with Klarić E et.al 2013 who evaluated the effects of two in-office bleaching agents containing a high concentration of hydrogen peroxide on human enamel and dentin surface. The results revealed that after the bleaching procedure, enamel and dentin surface showed changes on surface smoothness with slight irregularities. Also, reduction of surface microhardness was revealed. [9] Moreover, POGGIO C. et.al.2015 evaluated the morphology of the enamel surface following the application of the 35% of hydrogen peroxide, the bleached enamel showed pronounced surface changes and irregularities. [14] Other studies revealed alterations in the morphology of hard dental tissues in different degrees of severity, characterized by an increase in surface porosity, depressions, and superficial irregularities. [19,20] While, other researchers have reported contrary findings to the present study, Cakir FY. et.al. evaluated the surface morphology of the enamel following three different concentrations of at home bleaching agents, all of them showed no harmful changes on enamel and dentine surfaces. [21] Contemporary bleaching systems are based mostly on HP or one of its precursors, especially carbamide peroxide (CP), and these are often used in conjunction with an activating agent such as heat or light. [22] CP does break down to produce HP and urea, the resultant concentration from a 10% CP is only 3.35% HP. [23] The present study used an at home Zoom with 22% carbamide hydroxide which equivalent to 7.37% HP, this could explain the slight etching effect of Zoom on the enamel surface. In order to prevent and treat the possible adverse effect of bleaching and to restore the mineral loss, various methods of remineralization of bleached enamel have been used. In this study, it has been evaluated the possible protective effect of two different agents on bleached enamel, ACP paste, and MI Paste Plus. Amorphous calcium phosphate gel-like ACP is known to be an important factor for enamel remineralization. Amorphous calcium phosphate helps restoring the essential mineral balance in the mouth in an easy and efficient way and decrease adverse effects of tooth bleaching. [24] While MI Paste Plus™ is a sugar-free, water-based cream paste which was introduced to the American

market in April 2007. The active ingredient of MI Paste Plus™ is casein Phosphopeptide-amorphous calcium phosphate with 900ppm Fluoride (CPP-ACPF). [(12)] In this study, the application of the protective agents enhanced the creation of a superficial mineral layer. As demonstrated by scanning electron microscopy, this protective layer was more homogeneous for the specimens treated with MI Paste Plus (group 4) than in ACP group. Our results are in accordance with Borges et al. who showed significant reduction of microhardness after application of the acid bleaching agent, while the microhardness was enhanced by the application of fluoride gel on bleached enamel. [(25)] ACP Relief gel used in the current study also includes sodium fluoride as its active component. The results of our study were in partial agreement with Klarić E et.al 2013 who found an increase of surface microhardness and closed dentinal tubule by mineral deposition following application of ACP gel with artificial saliva storage of bleached enamel surfaces. [(9)] Moreover, it has been reported by Cunha et al., 2012 that the negative alteration of bovine enamel after bleaching was enhanced by the application of CPP-ACP (MI Plus Paste) either before or before and after bleaching procedure. [(26)] In addition, Poggio et al., 2013, concluded that the application of CPP-ACP (MI Plus Paste) could prevent the erosion of enamel and dentine by soft drinks. [(27)] In the present study, SEM observations revealed that the remineralization with CPP-ACP (MI Plus Paste) was better than the use of ACP (ACP relief gel) alone. These results were confirmed by using the DIAGNOdent to measure the mineral contents in the tested groups. The Amorphous Calcium Phosphate (ACP) systems are composed of unstabilized calcium and phosphate ions, were both phosphate salts (e.g., potassium phosphate) and calcium salts (e.g., calcium sulfate) are delivered individually intraorally. [(28)] As the salts combine with saliva, they dissolve, releasing calcium and phosphate ions, which will be precipitate immediately as ACP or amorphous calcium fluoride phosphate (ACFP) in the present of fluoride ions. However, these particles are not stable. [(29)] In 2016, Mirela B. et.al. investigated the effect of Ca2+ and F- incorporated in 45% carbamide peroxide bleaching on enamel minerals content using DIAGNOdent, they concluded that there is mild remineralization effect as a result of incorporated ions in the bleaching agent. [(15)] In order to stabilize the calcium and phosphate ions, the stabilizing proteins have been used such as casein in milk [(30)] and statherin in saliva [(31)] based on biomimetic casein Phosphopeptide as in CPP-ACP (MI Plus Paste). As a result, CPP-ACP (MI Plus Paste) will offer a higher reservoir of bioavailable calcium and phosphate ions in comparison with ACP only; [(29)] This could explain why the CPP-ACP (MI Plus Paste) showed better remineralization than ACP (ACP relief gel) alone in present study. These finding were confirmed with POGGIO C. et.al.2015, who tested the efficiency of different remineralizing agents (Remin Pro, Tooth Mousse, MI Paste Plus, and Profluorid Varnish) on bleached enamel surface and they concluded that the use of CPP-ACP (MI Plus Paste) showed complete and homogeneous protective layer which was better in remineralization than the other tested agents. [(14)] Clinically, the mineral intake would be faster because of the presence of saliva, plaque control and the use of fluorides. It has been noted that most studies evaluating the effect of the bleaching agents on the teeth have been in vitro. So further, in vivo evaluation is needed to be representative of the in vivo situation.

The use of the protective agents tested can be useful in clinical practice to reduce the adverse effects on enamel surface after bleaching procedures. Under the limitations of the present in vitro study, application of the protective pastes following bleaching is effective on repairing enamel surface morphology. However, the protective effect of MI Paste Plus product demonstrated a higher efficacy as compared to ACP relief gel product.

Approval of the study protocol: The Research Ethics Committee at the Faculty of Dentistry, Umm Al- Qura University has approved our study protocol from the ethical point of view in November 2016.

Ethical disclosures: The authors announce that no experiments were performed on voluntaries or animals and no data were collected from patient in this research.

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