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Effects of 35% Versus 10% Carbamide Peroxide At-Home Bleaching Agents with or without MI Paste Plus on Enamel Properties

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Key words
35%CP, at-home bleaching, MI paste plus, microhardness.

Abstract

This study compared the effects of 35% with 10% carbamide peroxide (CP) home used bleaching agents on enamel color change, microhardness, and surface changes in association with MI paste plus (CPP-ACPF). Forty buccal and lingual enamel samples from twenty human third molars were prepared and divided into four groups (n=10). The groups were bleached for two weeks according to different bleaching regimens as follow; G1 bleached with 10%CP, G2 bleached with 35%CP, G3 bleached with a mixture of 10%CP and MI paste plus, and G4 bleached with a mixture of 35%CP and MI paste plus. Color change was recorded with a colorimeter before and after bleaching and total color differences (ΔE*) was calculated. Vickers microhardness test was recorded for all the samples before and after treatment. One sample from each group was evaluated for surface changes by scan electron microscopy (SEM). The data were analyzed with repeated measure of variance and student's t-test at $p<0.05$. No significant difference in ΔE* was recorded among the four groups ($p>0.05$). Significant reduction in enamel microhardness was only recorded for G2 ($p=0.003$). SEM for G3 and G4 showed obvious mineral deposition. Similar teeth color whitening can be resulted after two weeks of bleaching with both 10%CP and 35%CP when used as recommended by the manufacturer with and without the addition of MI paste plus. Using 35%CP decreased enamel microhardness significantly, whereas MI paste plus when mixed with both bleaching agents could compensate for the microhardness reduction.

Introduction:

The first article on the use of 10% carbamide peroxide (CP) bleaching agent within a night guard to bleach vital teeth was published in 1989 by Haywood & Heymann [1]. This technique has become widely accepted nowadays and known as at-home bleaching technique. Since then, many published studies have further proven the efficiency and safety of this technique compared with other vital teeth bleaching techniques [2]. This fact encouraged manufacturers to market higher concentrations of at-home bleaching agents ranging up to 35%CP to be used for a shorter period of time within the bleaching tray. However, higher concentrations of bleaching agents may jeopardize enamel and dentin structures [3]. Physical and chemical changes of enamel surface after vital teeth bleaching are of a major concern to dentists as well as patients. Several researchers have proposed that bleaching treatments may be associated with changes in enamel

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microhardness, decrease calcium and phosphorus concentrations and increasing of surface roughness [4,5]. However, controversial results were published concerning the influence of different concentrations of bleaching agents on enamel surface. Some researchers have reported that longer contact time of bleaching gels even with low peroxide concentration such as 10%CP may negatively affect the enamel surface [6,7]. However, others proposed that bleaching gels with a relatively higher concentration produced more intense side effects compared with 10% CP [8]. To overcome the potential side effects related to the use of bleaching gels on the tooth surface, attempts have been adopted by manufacturers by adding minerals to the bleaching agents such as fluoride and/or calcium ions [9]. On the other hand, several researchers have investigated the effect of remineralizing pastes such as: MI paste (casein phosphopeptide-amorphous calcium phosphate; CPP-ACP) on bleached teeth surfaces[10-13]. According to the manufacturers, this agent contains CPP-ACP; an active ingredient which is a special milk-derived protein that has the ability to release bio-available calcium and phosphate to tooth surfaces [9]. Basically, these agents are topically applied to remineralize decay-damaged teeth by replacing lost calcium and phosphate [14]. Researchers have proposed that post operative application of CPP-ACP for two weeks on bleached enamel surfaces is able to decrease the bleaching agents' related adverse effects [10,11]; reestablishing the baseline enamel hardness and the reduced modulus of elasticity [7]. Most of these studies have used high concentrations of hydrogen peroxide (HP) and CP bleaching gels used for in-office bleaching technique. A recent study investigated the effect of MI paste plus (CPP-ACPF; a 10% CPP-ACP enhanced with 900 ppm fluoride) after in-office bleaching on enamel surface [14]. Two studies have investigated the effects of blending CPP-ACP with two concentrations of home used bleaching agents (10%CP & 16%CP) on enamel color change and microhardness [12,13]. However, the use of a high concentration bleaching agent such as 35%CP, as at-home bleaching agent, although for a short period of time (30 minutes a day), may still bring some concerns about its potential side effects on the tooth structure since its use should be consecutively continued for about two weeks. The effect of 35%CP home used bleaching agent on enamel properties in association with the use of MI paste plus has not been fully investigated. Therefore; the aim of the current *in vitro* study was to evaluate the effects of 35%CP compared with10%CP at-home bleaching agents with or without CPP-ACPF on enamel whitening, microhardness, and surface changes. Thus, the null hypotheses tested in the current study were: 1) There is no difference in teeth color whitening with the use of both bleaching agents alone or in association with CPP-ACPF and 2) There is no difference in enamel microhardness after applying 10% or 35% CP with or without the use of CPP-ACPF.

Materials and method:

Twenty human third molars were collected from surgical department. Teeth were cleaned and polished with pumice and the roots were cut about 1mm below the cementoenamel junction. The crowns of the teeth were cut mesiodistally to produce forty buccal and lingual enamel samples. Then, each sample, with the enamel surface exposed, was individually embedded in a plastic mould using chemically cured acrylic resin. The enamel surfaces were flattened using aluminum oxide abrasive papers with 600, 800, and 1200-grit. The specimens were then divided into four groups (n=10) according to the bleaching regimens.

Bleaching regimens

The bleaching treatment was performed for two weeks for all of the four groups. 10 %CP and 35%CP at-home bleaching agents (Opalescence PF, Ultradent Products, USA), were applied according to the manufacturer's instruction. MI paste plus (CPP-ACPF, GC, Ultradent Products Inc.) was mixed with the two bleaching agents and used as follow:
G1: treated with 10% CP for 8 hours/day.

G2: treated with 35% CP for 30 minutes/day.
G3: treated with a mixture of 10% CP and CPP-ACPF for 8 hours/day.
G4: treated with a mixture of 35% CP and CPP-ACPF for 30 minutes/day.

During the bleaching procedure of G1 and G2, about 0.5 to 1 mm thick of the bleaching agent was applied on the enamel surfaces for the required period of time. For G3 and G4 the bleaching agent was mixed with an equal amount of CPP-ACPF paste and adopted on the enamel surface. The application of the mixture was performed by the same operator to ensure applying equal amounts of the mixture every day. By the end of the daily bleaching period, the gel was first wiped off with damp cotton and then placing the samples under running water for 15 seconds. After that, all the specimens were stored in normal saline for the rest of the day.

Color measurements

Teeth color measurements, before bleaching (T0) and after bleaching (T1), were recorded objectively using a colorimeter (VITA Easyshade, Zahnfabrik; H. Rauter GmbH & Co, KG, Bad Sackingen, Germany). Color measurements were evaluated according to the CIE L*a*b* parameters established by the Commission International de l´Eclairage in 1976 [15]. The three-dimensional color coordinates; L*, a*, and b* represented; the lightness, shade and saturation in the red-green axis, and saturation in the blue-yellow axis, respectively. Total color difference (ΔE*) between before (T0) and after bleaching (T1) measurements was calculated as follow:

$$\Delta E^* = [(L^*1 - L^*0)2 + (a^*1 - a^*0)2 + (b^*1 - b^*0)2]\ 1/2$$

Microhardness measurements

All the four groups were measured for microhardness before the bleaching treatment (T1) and by the end of the treatment (T2). Vickers microhardness measurements were performed on the enamel surfaces by Innovatest (Vickers hard testing machine 4300, Hoffman group, Germany) with indentation load at 300g for 5 seconds. Three indentation loads were performed and the average of the three readings was adopted for each measurement.
The results were analyzed using SPSS program (SPSS 19.0 for windows, USA). Student's t-test was used to evaluate the color change between the groups. Repeated measure of variance (ANOVA) and pairwise comparisons were used to compare measurements of microhardness before and after the treatment. The significance level of 0.05 was adopted for all the tests.

Scan election microscopy (SEM)

One sample from each group was arbitrary selected and analyzed for surface changes. The specimens were dried, fixed on aluminum stubs and sputter-coated with gold. Enamel surface examination was performed with SEM (Angstrom advanced Inc., AIS2300C, Massachusetts, USA). Two SEM images for each sample were saved at 500 x and 1000 x magnifications.

Results:

Table 1 shows the means (SD) of ΔL*, Δa*, Δb*, and ΔE* for all the groups. No significant differences in all of the three color parameters (ΔL*, Δa*, and Δb*) were recorded for all groups ($p>0.05$). Means of ΔE* of the four groups were; 11.24, 10.89, 8.41, and 9.13, respectively. No significant difference in ΔE* was recorded among the four groups ($p>0.05$).
Table 2 represents microhardness measurements for all the four groups before (T1) and after bleaching (T2) treatment. Microhardness was reduced for G1; however, it was not significantly different from the baseline recordings. Significant difference was recorded for G2 ($p=0.003$). Both G3 & G4 showed no significant reduction in microhardness compared with the baseline measurements ($p>0.05$).
SEM photographs for the four samples of each experimental group are represented in Figures (1-4). Figures 1 & 2 showed

minor surface irregularities which were more evident for those bleached with 35%CP (Figure 2). While both figures 4 & 5 showed obvious mineral precipitations on the enamel surfaces.

Discussion:

With the increase marketing of new vital teeth bleaching products, *in vitro* and *in vivo* studies are still needed to support their safety and efficacy. It is well accepted that safety is of a prime concern when it comes to cosmetic procedures such as vital teeth bleaching. In respect to the two main dentists' supervised bleaching techniques; professionally administered (in-office bleaching system) and professionally dispensed (at-home bleaching system), both have proven effectiveness in teeth whitening [16]. For at-home bleaching technique, a variety of concentrations of carbamide peroxide bleaching agents are available ranging from 10%, 15%, 16%, 20%, and up to 35%CP. Different concentrations of CP break down into different concentrations of HP and urea. 10%CP breaks down into 3.5% HP, whereas, 35%CP produces about 10.6%HP[5]. Carbamide peroxide bleaching gels also contains carbopol which is added to decrease the releasing time of HP, hence extending the bleaching potential over a longer period of time. However, both concentration and time of exposure for CP bleaching agents have a major influence on the efficacy of teeth whitening as well as their side effects [17]. There are available evidences that support the safety of using 10%CP bleaching agent used for at-home bleaching treatment; however, concerns are still present with the use of at-home bleaching agents with higher HP concentrations[18].The results of the present study recorded comparable color change for all four groups. ΔE* values were 11.24, 10.89, 8.41, and 9.13, respectively. No significant differences in ΔE* was recorded whether between 10% and 35%CP alone or when they were blended with the remineralizing agent. Hence, the first null hypothesis tested in the present study should be accepted for all groups. Similar results were reported by Cvikl et al. [6] in which no color change differences were recorded between high concentration (35%CP) and low concentration (10%CP) bleaching agents immediately after the bleaching period. In the present study, CPP-ACPF did not affect color change produced with both 10% and 35% CP. Similar results were reported by Borges et al. when blended 10%CP & 16%CP at-home used bleaching agents with CPP-ACP [12]. In the literature, no consensus was reported on the effect of using bleaching gels with low concentration for a long contact time or high concentration for a short contact time on the physical properties of bleached enamel. Side effects such as decrease of enamel microhardness and surface changes have been widely studied by researchers to verify the influence of different types of bleaching agents. However, controversial results were still reported on the influence of different concentrations of home bleaching agents on enamel microhardness and surface change [19]. The results of the current study recorded significant decrease in enamel microhardness after bleaching with 35%CP alone but not for those bleached with 10%CP. Therefore, rejection of the second null hypothesis should be considered for G2 and accepted for G1. Such a result is in consistence with the results of recent studies reported by Klaric et al. [10] and Dey et al. [20]. They concluded that 10%CP can decrease enamel microhardness, however, not significantly. Soares et al. [8] stated that bleaching agents with higher concentration (16%CP) can produce more side effect than lower concentration (10%CP). However, such a result is not in accordance with those reported by Cvikl et al. [6] as they concluded that bleaching gels with a relatively high concentration of peroxide and shorter application time might be less harmful to enamel than agents with lower concentration and longer contact time. Several researchers have evaluated the influence of application of MI paste on the enamel surface after the bleaching regimens [7,10,11]. However, using these agents separately after the bleaching treatment for additional two weeks needs

more time and compliance by the patients. While, blending remineralizing agents with the bleaching gels to be used within the bleaching tray is more convenient to the patients. Besides, their use may decrease any expected tooth sensitivity during the bleaching regimen [9]. In this study, both 10% and 35%CP when blended with MI paste plus resulted in no statistically significant decrease in enamel microhardness compared with the baseline values. Such results require the acceptance of the second null hypothesis for G3 and G4. The use of CPP-ACPF in association with the used bleaching agents produced beneficial effect in reducing the negative influence of these agents on enamel microhardness. Similar results were recorded by Cunha et al. when used CPP-ACP before and after bleaching [11]. However, other studies reported that remineralizing agents when blended with the bleaching agents [12,13] or used after the bleaching treatment[14]could increase microhardness of bleached enamel. Such inconsistency with the results of the present study may be related to the use of artificial saliva as a storage media during the bleaching procedure by those studies. In the present study the samples were stored in normal saline in order to eliminate any potential remineralization effect that could be attributed to the storage media. Attin et al.[19] in their review of the effect of bleaching agents on enamel microhardness reported that the use of artificial saliva in *in vitro* or human saliva in *in situ* studies may highly affect the results. These storage media may reduce the risk of decreasing enamel microhardness due to bleaching treatments, by the remineralization action of saliva. Following enamel demineraliz-ation by the bleaching agents, a greater absorption of minerals by ionic exchange is produced replacing those lost during bleaching [21]. Analysis of SEM photographs of the enamel surfaces bleached with both CP bleaching agents showed minor morphological changes (Figures 1 & 2) which were more obvious for those bleached with 35%CP. These results are in consistent with those reported by other studies [5,13] showing areas of shallow erosions on the enamel surfaces. Figures 3 & 4 clearly identified mineral precipitations on the enamel surfaces for those groups treated with a mixture of bleaching agents and CPP-ACPF. Similar results were reported with other studies supporting that granules accumulations induced by remineralizing agents could protect the bleached enamel surface from potential morphological changes [13,22]. It has been proposed that microporosities formed by the bleaching agents provide susceptible areas for re-deposition of the remineralizing agents, similar to that which occurs in arrested caries[14]. For at-home vital teeth bleaching system, 35%CP bleaching agent is the highest concentration with the shortest application time used within the bleaching trays. Its use is convenient for those patients unable to wear the bleaching trays overnight with 10% CP. However, in respect to the safety issue, it is recommended to be used with a remineralizing agent to overcome the potential side effects to the enamel surface. Further clinical researches are still needed to evaluate other side effects such as teeth sensitivity that might be associated with these agents.

Conclusions:

Within the limitation of the current *in vitro* study, it can be concluded that:

1. Similar teeth color changes can be achieved after two weeks of bleaching with 10% and 35%CP when used as recommended by the manufacturer's instructions with and without the addition of MI paste plus (CPP-ACPF).

2. Enamel microhardness was significantly decreased after home bleaching with 35%CP but not with the use of 10% CP.

3. MI paste plus when mixed with home used 10%CP and 35%CP bleaching agents can compensate the microhardness reduction of bleached enamel associated with the use of these bleaching agents.

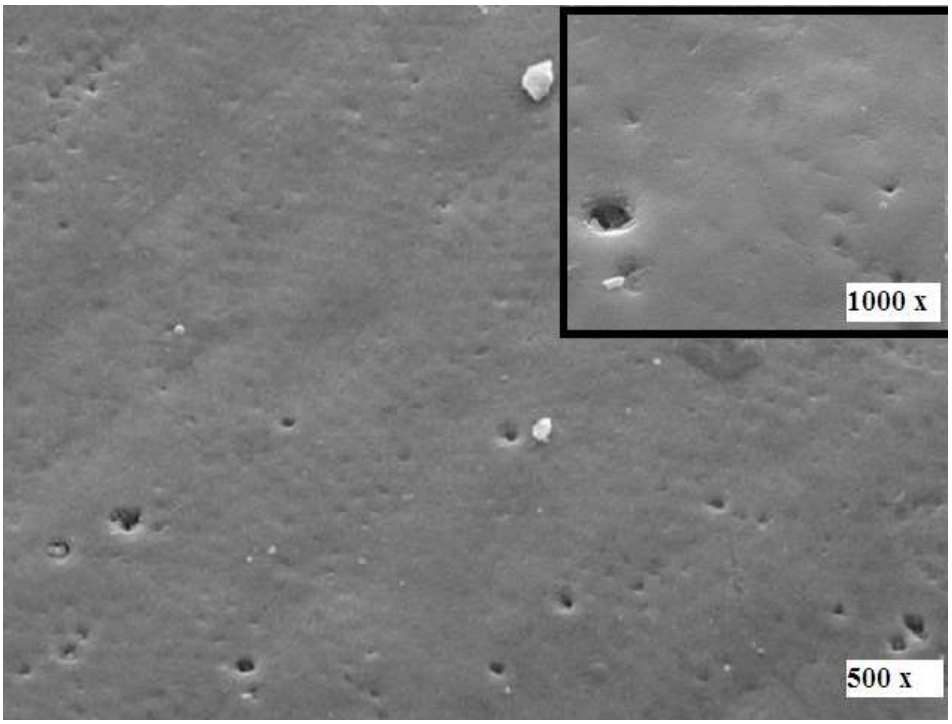

Fig. (1): SEM micrographs for enamel sample bleached with 10%CP showing minor changes. (1000 x and 500 x magnifications).

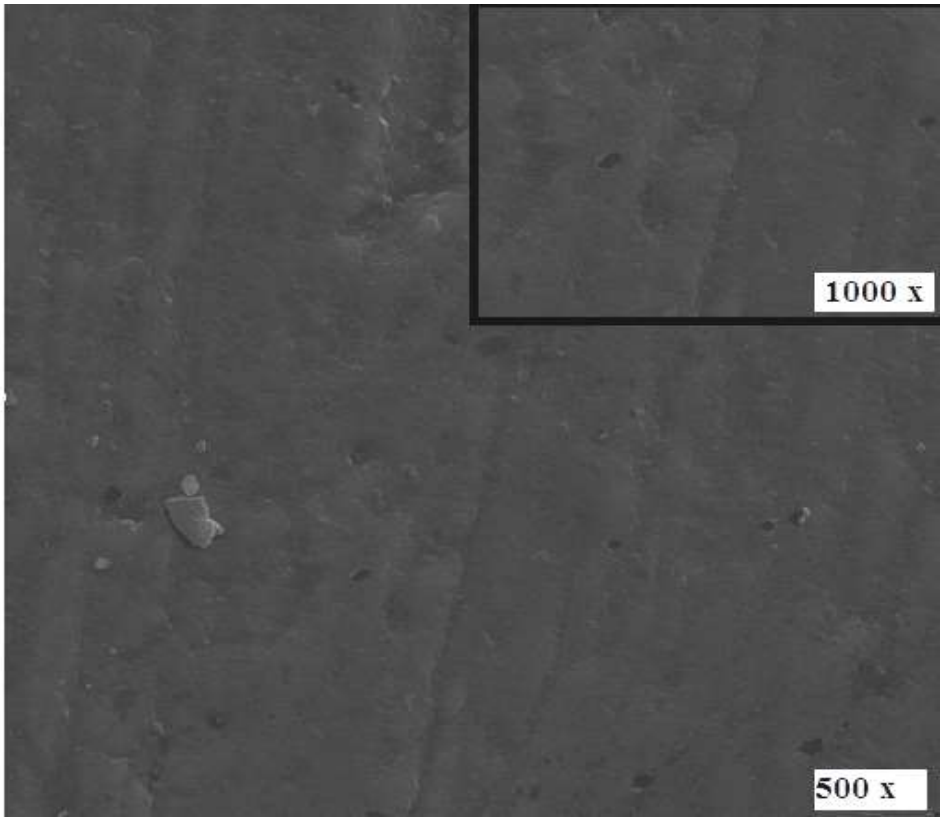

Fig.(2): SEM micrographs for enamel sample bleached with 35%CP showing shallow irregularities. (1000 x and 500 x magnifications).

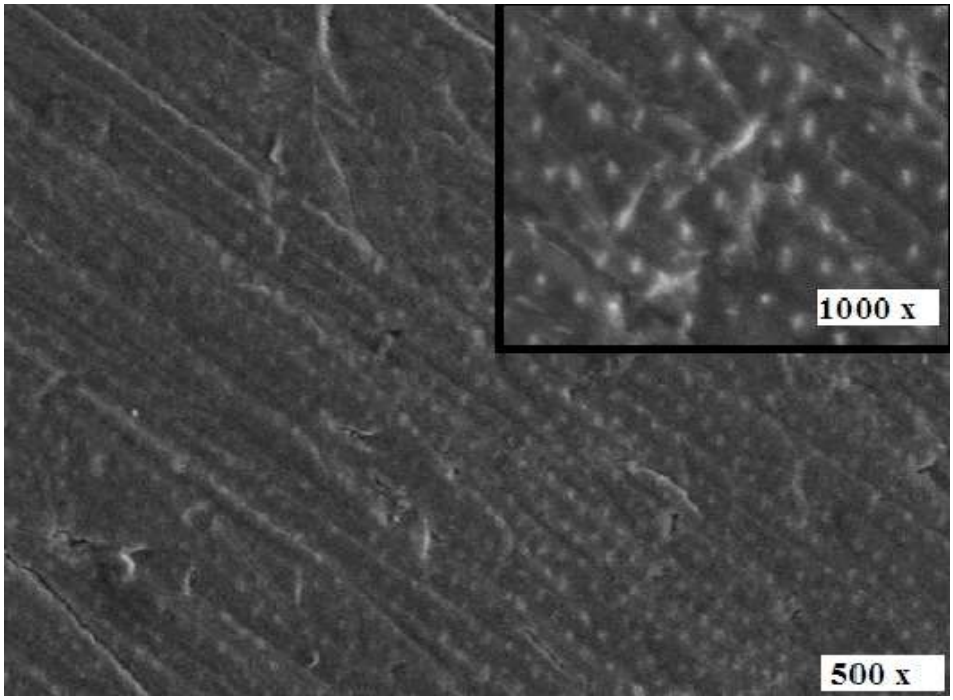

Fig.(3): SEM micrographs for enamel sample bleached with a mixture of 10%CP and CPP-ACPF showing precipitations of minerals. (1000 x and 500 x magnifications).

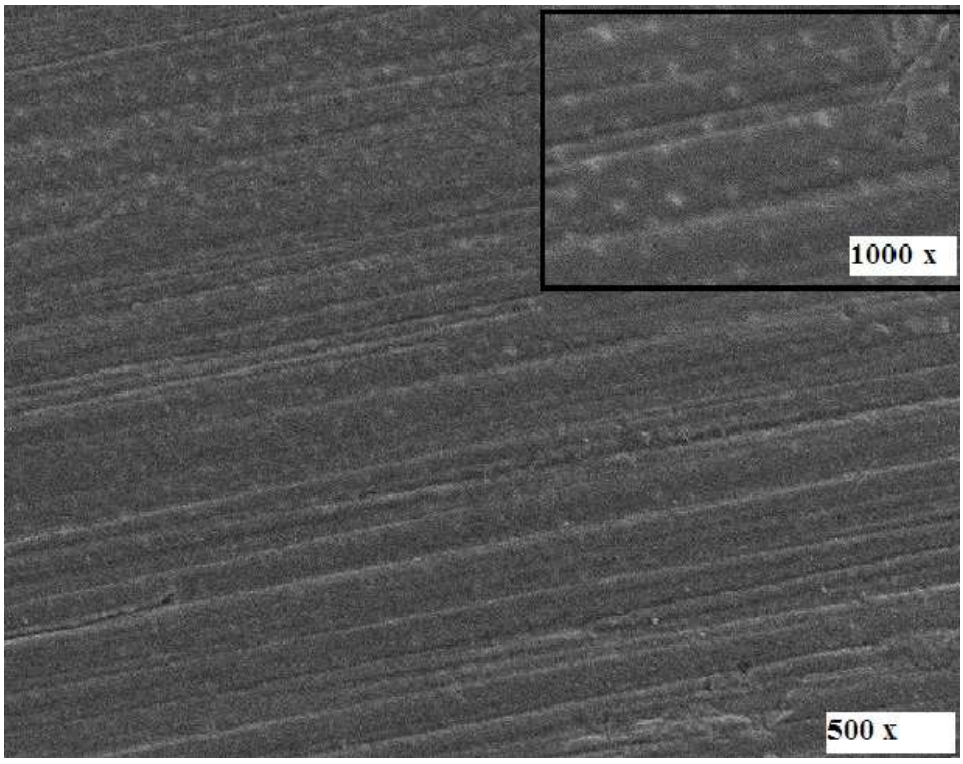

Fig.(4): SEM micrographs for enamel sample bleached with a mixture of 35%CP and CPP-ACPF showing precipitations of minerals. (1000 x and 500 x magnifications).

Table 1: Means (SD) of ΔL*, Δa*, Δb*, and ΔE* for all groups.

Groups	ΔL*	Δa*	Δb*	ΔE*
G1	6.82(2.01)	-1.48(0.76)	-8.04(3.84)	11.24 (1.75)
G2	6.14(3.51)	-1.62(0.71)	-7.36(4.38)	10.89 (1.35)
G3	6.16(2.07)	-1.00(0.93)	-4.84(2.78)	8.41 (1.57)
G4	5.44(2.52)	-0.66(0.76)	-6.98(1.46)	9.13 (1.82)

Table 2: Means (SD) of enamel microhardness before (T1) and after (T2) surface treatment for all groups.

Groups		Mean*	(SD)
G1	T1	277.66	32.38
	T2	263.00	13.88
G2	T1	330.08 (**a**)	24.55
	T2	296.76 (**a**)	14.36
G3	T1	297.84	17.46
	T2	290.96	20.94
G4	T1	292.46	27.97
	T2	283.62	35.44

*Mean values with similar lower case letters are significantly different at $p<0.05$

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