

Effect of Remineralization on Color Change of Bleached Tooth

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Abstract

The aim of this *in vitro* study was to investigate the effect of remineralization and time on the color and microhardness change of bleached teeth using 10 % carbamide peroxide (CP) and 40 % hydrogen peroxide (HP). Seventy-two 6x6x2 mm³ enamel slabs were prepared from human premolars. The specimens' color and Vickers' microhardness were recorded at baseline (T0). The specimens were divided into six groups: twelve specimens per group. Two groups were treated with 10 % CP with or without casein phosphopeptides and amorphous calcium phosphate (CPP-ACP) (10 % CP and 10 % CP/CPP-ACP, respectively), two groups were treated with 40 % HP with or without CPP-ACP (40 % HP and 40 % HP/CPP-ACP, respectively), one group received no bleaching (CON) and another group received only CPP-ACP treatment without bleaching, (CON/CPP-ACP). The CPP-ACP groups were treated with CPP-ACP twice daily for five minutes for seven days after completing their respective protocol. The color change and Vickers' microhardness were recorded at three time points after treatment; one day (T1), two weeks (T2), and one month (T3). After one month, the tooth color changed (ΔE) in all groups at every time point; however, significant ΔE was found only at T3 for the 10 % CP and the CON/CPP-ACP groups ($p < 0.05$). Microhardness tended to decrease from the baseline (T0) value at each time point. There were significant differences between time points in microhardness (ΔVHN) in the 40 % HP, CON, and CON/CPP-ACP groups. A relationship between ΔE and ΔVHN was found in the 10 % CP and 10 % CP/CPP-ACP groups. In summary, tooth whiteness decreased in the 10 % CP group from T1 to T3. CPP-ACP can prevent color relapse in the 10 % CP/CPP-ACP and CON/CPP-ACP groups. Microhardness improved in the 10 % CP/CPP-ACP group compared with the 10 % CP group at T3. Both tooth whiteness and microhardness were stable for at least one month when CPP-ACP was used as an intervention in the 10 % CP/CPP-ACP group.

Keywords: Color relapse, Tooth bleaching, Remineralization, CPP-ACP

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Introduction

Over a person's lifetime, their teeth can become stained by various means, which can be considered unesthetic. Patients often request the dentist to improve their tooth color. To address this issue, hydrogen peroxide (HP) and carbamide peroxide (CP) have been used for decades for in-office tooth bleaching and home-applied bleaching, respectively.¹⁻³ However, the color of bleached teeth is not stable, returning to a darker or yellower color over time.⁴⁻⁵ Studies have demonstrated that the development of a darker or more yellowish tooth color after bleaching resulted from the absorption of external organic pigments and stains from various food into the tooth structure or dental restorations.⁶⁻⁸

Bleaching caused changes in the morphology of the enamel surface due to demineralization, and reduced enamel surface hardness and fracture toughness.^{9,10} Previous studies have reported that both in-office and home-applied bleaching caused tooth demineralization, which was confirmed by microhardness, micro-computed tomography, or atomic absorption spectrophotometry evaluation.¹¹⁻¹⁴ Tooth color becomes lighter after bleaching due to the destruction of pigments by peroxide and demineralization.¹⁴ Therefore, remineralization after bleaching may affect color relapse in bleached teeth because strength and color value both change after bleaching.

Villalta *et al.* and Um *et al.* investigated extrinsic factors, such as the absorption of stains into dental restorations, finding that extrinsic staining by wine and coffee caused significant discoloration.⁶⁻⁸ Other studies found that if the tooth had higher initial color lightening, tooth color relapse was more likely to occur after one month.¹⁵⁻¹⁶ Moreover, the degree of relapse corresponded with the degree of initial color change.¹⁶ Tooth color was stable for six months after performing two in-office treatment sessions.¹⁷ However, after in-office bleaching, tooth whiteness decreased within one hour. Thus, the initial whitening of the tooth was considered to be

mainly due to the effect of dehydration on the initial color change.^{4,18,19}

Spectrophotometer is the highest reliable standard for color matching.^{4,20,21} Vita Easyshade (Vita Zahnfabrik, Bad Säckingen, Germany) is a spectrophotometer, which measures a small spot area approximately 5 mm diameter from the focal tip. This device translates data into both of Vita shades and also into CIELAB data (L*,a*,b*) from halogen light reflectance and scattered light.²²

The microhardness test is one of the most accurate evaluations for tooth remineralization. Vicker's hardness is used for small specimens by a diamond indenter that creates a light loads indentation, which ranges from a few grams to one or several kilograms. The opposing indenter faces are set at a 136-degree angle from one another.^{10,23-24}

Casein phosphopeptides and amorphous calcium phosphate (CPP-ACP), has demonstrated effects on remineralization, tooth sensitivity, flexural strength, and staining after bleaching.²⁴⁻²⁷ A study showed that there was no difference between the influence of CPP-ACP treatment and natural saliva on tooth remineralization after fifteen days.²⁸ In contrast, another study showed that CPP-ACP applied for one minute, significantly increased mineral deposition and decreased carious lesion depth.²⁹

Currently, how to prevent color relapse is not well understood, and there are few studies regarding this issue. The null hypotheses in this study were that there was no correlation between microhardness change and color change, and that there was no difference in bleached color at any evaluated time point when teeth were bleached with or without CPP-ACP. The objective of this study was to determine if remineralization, using CPP-ACP affected the color change and microhardness in the teeth after bleaching.

Materials and Methods

Specimen preparation

The required sample size was determined after a pilot study. Extracted human upper and/or lower premolars were collected after informed consent, and obtained under a protocol approved by the Ethics Committee of the Faculty of Dentistry, Chulalongkorn University, (HREC-DCU 2016-063). The selected teeth were disinfected in a 0.1 % thymol solution (M Dent, Bangkok, Thailand) for a period of six months according to ISO 28399 and 11405.

External staining was removed by polishing each tooth with pumices and a rubber cup. Any teeth with visible cracks on the enamel surface, carious lesions, pathologic lesions or restorations were excluded from this study. All teeth were measured with Easyshade V at middle 1/3 of each tooth and selected only A and B shade. Seventy-two teeth were decoronated at the CEJ using a diamond disc 104918.190 HP (Diaswiss S.A., Nyon, Switzerland), and the crowns were sectioned buccolingually. The buccal crown portions were prepared with a diamond disc generating a 6x6x2 mm³ slabs. Subsequently, the surface of each specimen was flattened with 400 grit silicon carbide paper and polished

with a polishing machine (Nano 2000, Pace Technologies, USA) that used 800, 1200, 1600 grit silicon carbide papers (Leco Corporation, MI, USA) respectively, under approximately 25 N pressure, 100 rpm for each grit, and a constant water flow. Aluminum oxide paste with 2000 grit silicon carbide paper was used for the last step of polishing. To confirm that the surface of the specimens were on enamel, the surface and microhardness were observed on the computer screen of microhardness testing machine, which did not present dentinal tubule pattern on the surface.

Model preparation

A 6 mm diameter trephine bur (Hu-friedy, Chicago, IL, USA) was used to create two rows of 6 mm holes (6/plate) 5 mm apart in the plastic plates for the tooth specimens to be placed in (Fig. 1a). Twelve plates were prepared (2 plates/group). The specimens (n = 6) were placed in the cavities of each block (Fig. 1b). Clear epoxy resin and accelerator liquid (SC2B clear, Super Silicone & Resin Art, Bangkok, Thailand) were mixed and poured into the cavities up to the level of the specimen and was allowed to set for 24 hours.

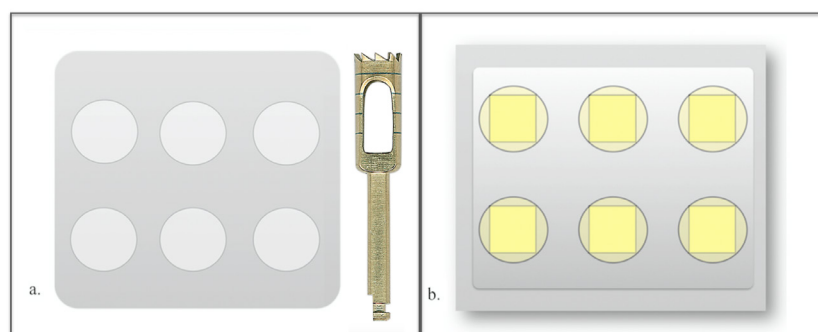


Figure 1 a) A plastic plate punched round holes and trephine bur. b) Tooth sections in their plastic template secured with epoxy resin.

Specimen treatment

The specimen plates were randomized into six groups (n=12/group). The color was measured with a spectrophotometer (Vita Easyshade V, Vita Zahnfabrik, Germany) and the microhardness of each specimen was

determined using a microhardness tester (Fm-700e Type D, Future-Tech, Japan) before treatment, as a baseline. The spectrophotometer was calibrated before each measurement and a calibrate mode was used 3 times to evaluate each time point. Subsequently, each treatment

group followed its respective protocol (Table 1). Two groups (10 % CP and 10 % CP/CP-ACP) were treated with 10 % carbamide peroxide (Opalescence PF 10 %, Ultradent, South Jordan, UT, USA) for eight hours a day for fourteen days and collected in artificial saliva at all times (Table 1, 2). Two groups (40 % HP and 40 % HP/CP-ACP) were treated with 40 % hydrogen peroxide (Opalescence Boost PF 40 %, Ultradent, South Jordan, UT, USA) for two sessions, twenty minutes per session, only one day after that collected in artificial saliva at all times (Table 1, 2). Only three groups (10 % CP/CP-ACP, 40 % HP/CP-ACP and CON/CP-ACP) were added CP-ACP treatment (Table 1, 2).

Whitening treatment using 10 % carbamide peroxide (Fig. 2a) and 40 % hydrogen peroxide (Fig. 2b) was performed according to the manufacturer's instructions. The 1 mm plastic plates with punched holes were used on the top of specimen plates to control the volume of each bleaching agent (Fig. 2a, 2b).

CP-ACP (Tooth Mousse, GC Europe N.V., Leuven, Belgium) was used as a remineralizing agent for two sessions continuously, five minutes per session after completing the bleaching treatment (10 % CP/CP-ACP and 40 % HP/CP-ACP) (Table 1, 2). The control group (CON) received no bleaching, and the remineralization control group (CON/CP-ACP) was only treated with CP-ACP (Table 1, 2). To control the volume of the bleaching agent and remineralizing agent, a 40x45x1 mm³ plate was placed over the plate containing the specimens and a 6-mm diameter hole was punched directly over each specimen. A cement spatula was used to remove excess CP-ACP. All of the remineralization procedures were repeated everyday for one week. The artificial saliva was refreshed every one to two days.

Color measurement

The color of each specimen was evaluated before treatment as baseline (T0) and ΔE was determined at one day (T1), two weeks (T2) and one month (T3)

after bleaching using a spectrophotometer (Vita Easyshade V, Vita Zahnfabrik, Germany) at the inner circle area shown in Figure 3. The spectrophotometer was calibrated before each evaluation as per the manufacturer's instructions. The calibrated mode was used to measure three times at each time point to collect the average data. Only CIE L*a*b color data was recorded to compare the color change on specimens.

Microhardness evaluation

Vickers' microhardness (VHN) evaluation was performed after color evaluation. The force load was 50 g for fifteen seconds, based on ISO28399: 2011. Each specimen was evaluated for microhardness three times with a distance of 100 μ m between each loading point. According to FM-800 manufacturer instruction, the minimum distance of each indentation was at least four times the diagonal length from the center of the indentation. The distance of indentation from margin of specimen was a minimum of 2.5 times of the diagonal length. The microhardness was evaluated at a different corner of the specimen at baseline (before bleaching) (T0), one day (T1), two weeks (T2), and one month (T3) after bleaching (Fig.3). Mean microhardness values were calculated from three indentations each time point.

After the color and microhardness evaluations, the specimens were stored in humidified chamber with artificial saliva at 37°C in the incubator (Contherm Scientific Ltd., Petone, New Zealand) until the next evaluation. The control groups were measured after two weeks of immersion in artificial saliva.

Statistical Analysis

All statistical analyses were performed using the software SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) with 95 % confidence interval. Normality of the data was confirmed using normal probability plots and the Kolmogorov-Smirnov test. Homogeneity of variance was confirmed using Levene's test. Regression analysis was conducted to analyze the correlation between

tooth color change (ΔE) and microhardness change (ΔVHN) in each group. The repeated measure test was used to determine the interaction between time and

ΔE , and the interaction between time and ΔVHN in each group after completing treatment. Descriptive statistics (mean and SD) were used to evaluate ΔE and VHN.

Table 1 The experimental groups based on treatment agent and application protocol.

Group	Treatment agent +Application protocol		Remineralizing agent +Application protocol	
1. 10 % CP	10 % Carbamide peroxide	8 h x 14 d	Artificial saliva	All the time
2. 10 % CP/CPP-ACP	10 % Carbamide peroxide	8 h x 14 d	Artificial saliva CPP-ACP	All the time 2 x 5 min x 7d
3. 40 % HP	40 % Hydrogen peroxide	2 x 20 min x 1 d	Artificial saliva	All the time
4. 40 % HP/CPP-ACP	40 % Hydrogen peroxide	2 x 20 min x 1 d	Artificial saliva CPP-ACP	All the time 2 x 5 min x 7d
5. CON (Negative Control)	Artificial saliva	14 d	Artificial saliva	All the time
6. CON/CPP-ACP (Positive Control)	Artificial saliva	14 d	Artificial saliva CPP-ACP	All the time 2 x 5 min x 7d

Abbreviations: CP = 10 % Carbamide peroxide treatment, CP/CPP-ACP = 10 % Carbamide peroxide treatment follow by CPP-ACP application, HP = 40 % Hydrogen peroxide treatment, HP/CPP-ACP = Hydrogen peroxide treatment follow by CPP-ACP application, CON = Negative control (no treatment), CON/CPP-ACP = Positive control (no treatment with CPP-ACP application), h = hours, d = days, min = minutes

Table 2 The materials used in this study.

Product Name	Materials/Type	Composition	Manufacturer
Opalescence PF 10 % Lot no. BCV8Y	10 % carbamide peroxide gel	10 % Carbamide peroxide, 0.5 % Potassium nitrate, 0.11 % Fluoride ion, Carbopol, Glycerin, flavor	Ultradent, S Jordan UT, USA
Opalescence Boost PF 40 % Lot no. BC25B	40 % hydrogen peroxide gel	Gel: 40 % Hydrogen peroxide Activator: Potassium hydroxide, 1.1 % Sodium fluoride and 3 % Potassium nitrate	Ultradent, S Jordan UT, USA
GC Tooth Mousse Lot no. 150303S	CPP-ACP cream	Pure water, Glycerol, CPP-ACP, D-sorbitol, CMC-Na, Propylene glycol, Silicon dioxide, Titanium dioxide, Xylitol, Phosphoric acid, Flavoring, Zinc oxide, Sodium saccharin, Ethyl p-hydroxybenzoate, Magnesium oxide, Guar gum, Propyl p-hydroxyben- zoate, Butyl p-hydroxybenzoate	GC Europe N.V., Leuven, Belgium
Artificial Saliva Lot no. 129/59, 168/59, 186/59	Spray	Xanthan gum, Sodium carboxymethylcellulose, Potassium chloride, Sodium chloride, Magnesium chloride, Calcium chloride, Potassium dihydrogen orthophosphate, Methyl p-hydroxybenzoate	Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

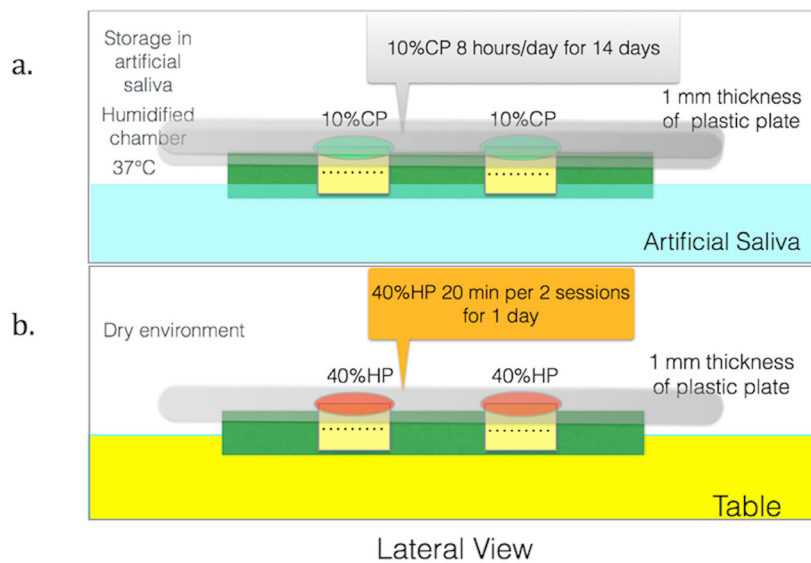


Figure 2 a) Specimens treated with 10 % carbamide peroxide in a humidified chamber to mimic oral environment (lateral view).
b) Specimens treated with 40 % hydrogen peroxide without artificial saliva to mimic the clinical procedure (lateral view).

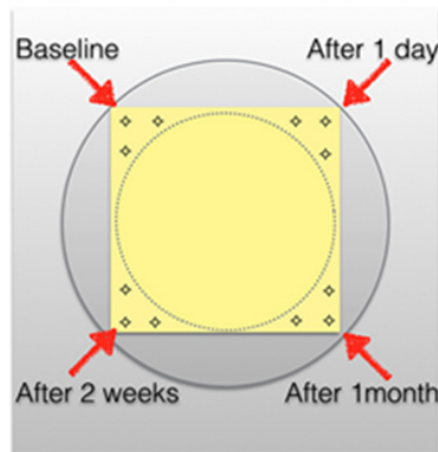


Figure 3 A tooth specimen in its plastic template. The arrows point to the locations of microhardness indentations at the four measurement time points. The circle is the area for color evaluation.

Results

Tooth color change is presented in Table 3 and Figure 4. The majority of color changes in the 10 % CP and 10 % CP/CPP-ACP groups were due to ΔL . Whereas Δb caused the majority of the color changes in the 40 % HP, 40 % HP/CPP-ACP, and two control groups (Fig 4).

ΔE decreased in the 10 % CP, 40 % HP and CON groups at T3 compared with T1. In contrast, the

groups treated with CPP-ACP (10 % CP/CPP-ACP, 40 % HP/CPP-ACP and CON/CPP-ACP) tended to demonstrate increased ΔE at T3 compared with T1 (Table 3). However, there were significantly different color changes only in the 10 % CP and CON/CPP-ACP groups at T3 ($p=0.039$ and $p=0.000$, respectively).

Mean microhardness (VHN) was presented in

Table 4. The VHN of 10 % CP group significantly decreased at T3 ($p<0.05$) (Table 4). The others groups were not significantly changed in VHN at the final time point T3 ($p<0.05$) (Table 4).

The correlation between Δ VHN and Δ E for each group is presented in Table 5. Only the 10 % CP, and the 10 % CP/CP-ACP groups demonstrated a correlation between Δ VHN and Δ E ($p=0.002$ and $p=0.003$, respectively) (Table 5).

Table 3 Mean and standard deviation in tooth color change (Δ E) in the groups at T1, T2 and T3 (T1=1 day, T2=2 weeks, and T3=1 month) after bleaching.

Type of Treatment	Tooth Color Change (Δ E) at 3 time points ($p<0.05$)		
	Mean \pm SD	Mean \pm SD	Mean \pm SD
	T1	T2	T3
10 % CP	8.83 \pm 0.60 ^a	8.15 \pm 0.84 ^a	7.53 \pm 0.76 ^s
10 % CP/CP-ACP	10.06 \pm 0.57 ^b	10.34 \pm 1.09 ^b	11.07 \pm 0.71 ^b
40 % HP	9.75 \pm 1.23 ^c	9.24 \pm 0.92 ^c	8.31 \pm 0.91 ^c
40 % HP/CP-ACP	7.58 \pm 0.93 ^d	7.05 \pm 0.89 ^d	8.10 \pm 0.62 ^d
CON (Negative Control)	2.98 \pm 1.04 ^e	3.78 \pm 0.63 ^e	2.56 \pm 0.31 ^e
CON/CP-ACP (Positive Control)	3.60 \pm 0.64 ^f	3.10 \pm 0.74 ^f	7.31 \pm 0.87 ^h

The same letter indicates no significant difference between groups in the same row at $p<0.05$.

Table 4 Mean and standard deviation (SD) in microhardness (VHN) between the groups at T0=baseline, T1=1 day, T2=2 weeks, and T3=1 month after bleaching.

Type of Treatment	Microhardness Value (VHN)			
	Mean (T0) \pm SD	Mean (T1) \pm SD	Mean (T2) \pm SD	Mean (T3) \pm SD
10 % CP	309.28 ^a \pm 35.39	284.21 ^a \pm 46.61	279.39 ^a \pm 16.48	278.17 ^s \pm 22.35
10 % CP/CP-ACP	287.96 ^b \pm 51.26	296.88 ^b \pm 35.79	300.91 ^b \pm 32.64	297.13 ^b \pm 27.23
40 % HP	272.36 ^{ch} \pm 39.81	317.66 ^{cl} \pm 33.00	327.90 ⁱ \pm 4.35	272.78 ^h \pm 20.45
40 % HP/CP-ACP	280.69 ^d \pm 45.47	301.40 ^d \pm 36.04	313.66 ^d \pm 22.80	304.73 ^d \pm 18.61
CON (Negative Control)	265.03 ^e \pm 34.70	313.49 ^j \pm 24.42	280.22 ^e \pm 19.95	269.50 ^e \pm 31.95
CON/CP-ACP (Positive Control)	295.33 ^f \pm 36.29	304.98 ^f \pm 31.94	307.92 ^f \pm 52.41	298.84 ^f \pm 22.53

The same letter indicates no significant differences between groups in the same row at $p<0.05$.

Table 5 Regression analysis indicated a relationship between Δ VHN and Δ E in each group.

Group	Standardized Coefficients Beta	t	p-value
10 % CP	0.493	3.303	.002*
10 % CP/CP-ACP	-0.479	-3.179	.003*
40 % HP	-0.279	-1.691	.100
40 % HP/CP-ACP	0.008	0.047	.963
CON (Negative Control)	0.108	0.634	.531
CON/CP-ACP (Positive Control)	0.015	0.087	.931

* Significant relationship between Δ VHN and Δ E in each group at $p < 0.05$ dependent Variable: Δ VHN, Predictor: Δ E

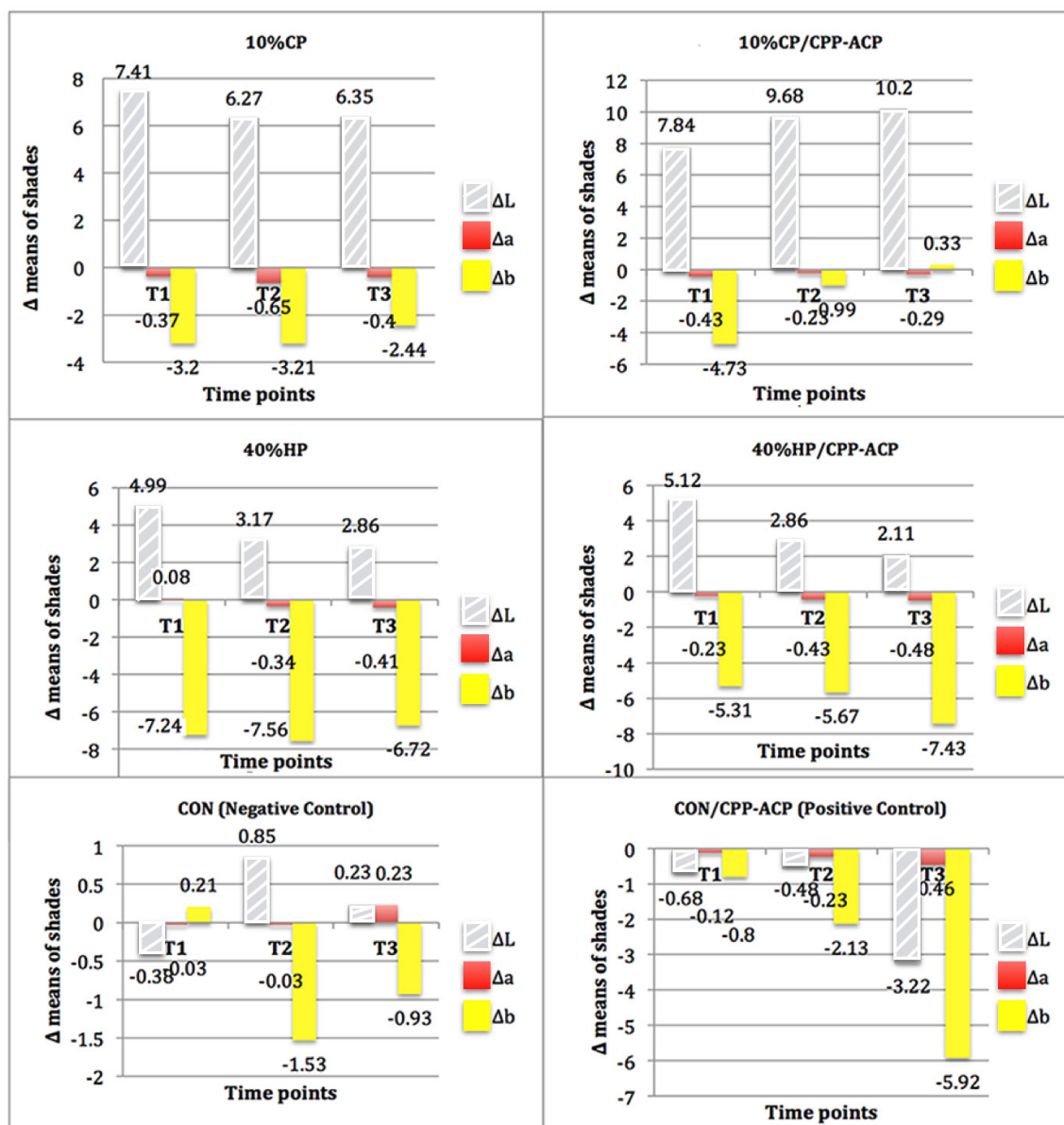


Figure 4 Mean ΔL , a , and b at three time points T1= 1 day, T2 = 2 weeks, and T3 = 1 month after bleaching in all groups.

Discussion

Previous studies found that carbamide peroxide used for home-applied bleaching, and hydrogen peroxide for in-office bleaching caused tooth surface demineralization.^{12,14,30,31} Therefore, the present study investigated whether tooth remineralization resulted in the color relapse of the bleached teeth. The microhardness results in the present study were similar to those of other studies, where the use of hydrogen peroxide (in-office

bleaching) did not result in a change in microhardness.^{23,32} Moreover, there were no significant difference in microhardness of the 40 % HP and 40 % HP/CPP-ACP groups after one month (T3). These results agree with another study in which the surface treatment with fluoride and CPP-ACP did not improve surface remineralization compared with fluoride treatment, suggesting that fluoride may hinder the effect of CPP-ACP.³³

In contrast to the in-office bleached teeth results, the microhardness in 10 % CP group decreased significantly at T3 compared with baseline; however, there was no significant difference in microhardness in the 10 % CP/ CPP-ACP group. This may be because CPP-ACP increased the surface microhardness of the home-bleached teeth compared with the in-office bleached teeth. The microhardness in the two control groups were also not significantly different after one month (T3). The microhardness in the 10 % CP and 40 % HP groups were different, the microhardness in the 10 % CP group decreased over time, while the microhardness in the 40 % HP group increased from T0 to T2, but decreased at T3. This may be because the higher percentage of fluoride contained in the 40 % HP bleaching gel was more effective in remineralizing the tooth surface by higher formation of many fluoroapatite crystal on tooth surface (Table 2).^{34,37} However, a recent study found that the use of 37.5 % hydrogen peroxide for one hour resulted in greater morphology change than that for thirty minutes and 16 % carbamide peroxide for fourteen and twenty-eight hours.³³

The pH of the home-applied bleaching gel used in the present study was lower than that of the in-office bleaching gel at 6.5 and 7, respectively as per the manufacturer's description. Some studies found that bleaching gels with a pH less than 5.2 caused demineralization.^{36,37} Although the pH of the bleaching gels were not less than the critical pH of approximately 5.5, which can cause tooth structure demineralization,³⁹ the microhardness in the home-bleached groups without CPP-ACP decreased over time.¹² This may be because the longer exposure times of the home-applied bleaching (8 hours/day for 14 days) compared with that of the in-office bleaching resulted in a more demineralized tooth surface.

In the present study, tooth whiteness of the 10 % CP group was reduced (decreased ΔE) at two weeks after bleaching, whereas, the 10 % CP/ CPP-ACP group did not demonstrate color relapse at any time

point, in fact, whiteness increased from two weeks until one month. These results indicate that, the use of CPP-ACP might have an effect on preventing color relapse in home-bleached teeth. For the in-office bleaching groups, the tooth color of the 40 % HP group was not significantly different compared with the tooth color of the 40 % HP/ CPP-ACP group at one month. Therefore, remineralization using CPP-ACP minimally affected the color of in-office bleached teeth. In the control groups, CON/ CPP-ACP group were whiter compared with the CON group after one month, and this change ($\Delta E = 3.71$) could be detected by the human eye (minimum visually detectable = 2.69-3.70).^{40,41} After one month (T3), groups treated with CPP-ACP, increased in whiteness (increased ΔE) by increasing ΔL or reducing Δb . Thus, CPP-ACP, in addition to its remineralization effect, may improve tooth whiteness and prevent tooth color relapse for at least one month in either normal or home-bleached teeth. This may be due to the whiteness and opacity of CPP-ACP that is deposited in the porous tooth structures during remineralization.

CPP-ACP was used in this study as a remineralizing agent. CPP-ACP, made from dietary products such as milk, and cheese, has been shown to possess an anticariogenic effect. Several studies showed that tooth remineralization using CPP-ACP strengthened the enamel surface.^{24-26,33,42} The use of CPP-ACP also prevented the re-staining of the bleached teeth.²⁷ Other remineralizing agents, such as topical fluorides, were not used in the current study because they are for professional use only and their toxicity is not conclusive. Artificial saliva is a remineralizing agent that mimics the properties of human saliva. A recent study concerning storage conditions found that natural saliva was the only storage media that showed similar behavior to in situ.⁴³ However, the use of both artificial saliva and natural saliva were efficient in enamel remineralization.⁴⁴ This study also present the result of remineralizing with artificial saliva as showed in microhardness result (Table 4).

Regression analysis demonstrated that

microhardness change and color change in the present study were correlated only in the home-applied bleaching 10 % CP and 10 % CP/CP-ACP groups (10 % CP: $p=0.002$, 10 % CP/CP-ACP: $p=0.003$). Thus, the first null hypothesis was rejected. For the 10 % CP group, beta was 0.493 indicating that tooth color and microhardness correspondingly reduced. For the 10 % CP/CP-ACP group, beta was -0.479, thus, when microhardness increased the tooth color became darker, or when microhardness decreased that the tooth color became whiter. These findings contradicted the color results because microhardness was reduced at T3 the opposite of the effect observed for color. The final microhardness in the other groups were also reduced (T2 to T3). However, duration of microhardness reduction was different from another study in that none of the remineralizing products maintained microhardness fourteen days post-bleaching.⁴⁴ In addition, there was no relationship between color change and microhardness change in the in-office bleaching groups and control groups ($p>0.05$). The fluoride level of in-office bleaching group was higher than that of home-applied bleaching for 10 times (10,000 ppm, 1,000 ppm respectively) (Table 2).⁴⁵ This may be the reason why there had no relationship between that of in-office bleaching groups, because the effect of 1.1 % fluoride ion or 10,000 ppm fluoride in both groups of 40 % HP was higher effective to stabilize enamel microhardness than that of the 10 % CP group (0.11 % fluoride ion or 1,000 ppm) in all periods.^{46,47}

The 10 % CP and CON/CP-ACP groups demonstrated a significant color change (ΔE) between time points (T1-T3) ($p<0.05$). Therefore the second null hypothesis was rejected. The number of samples treated did not allow them all to be evaluated immediately. Thus to eliminate differences based on time of evaluation, the color and microhardness evaluations in the present study were performed the day after bleaching. The spectrophotometer and microhardness tester were calibrated before each measurement. Both evaluations

were performed with the same investigator. The color measurement was performed with the Vita Easyshade V spectrophotometer for all groups using the same neutral grey background. The spectrophotometer tip abutted the specimens to eliminate light reflection from the environment into the sensor. Because Δb is the most visually perceptible parameter,⁴⁷ a significant improvement in Δb was found in the positive control group, which received CP-ACP treatment. The use of a high concentration of hydrogen peroxide for only one day may reduce enamel microhardness less compared with a low concentration of carbamide peroxide at a high frequency. Other bleaching gels may generate different results due to the different compositions and percentage of bleaching agents contained in each bleaching gel. However, CP-ACP application reduced enamel demineralization after frequent bleaching. The clinical implication from this study is that CP-ACP can be used to prevent tooth color relapse. The use of CP-ACP on a non-bleached tooth for at least seven days may enhance ΔE or be a lighter color. In-office bleaching (Opalescence Boost PF 40 %) may be suitable for reducing yellow scale (b^*). Home-applied bleaching (Opalescence 10 % PF) may be suitable for reducing black scale (L^*). The other remineralizing materials such as tri-calcium phosphate, bioactive glass, etc. should be evaluated in further studies to compare their effects on color change and remineralization. CP-ACP should be used for longer than seven days to evaluate the microhardness result in further studies.

Conclusion

Within the limitations of this study, it can be concluded that tooth color relapse was found within thirty days in the groups treated with 10 % CP ($p<0.05$). The final microhardness significantly decreased in the 10 % CP group after one month. Applying CP-ACP on home-bleached teeth prevents tooth color relapse and tooth strength reduction for at least thirty days.

Conflict of Interest

The authors did not receive any products, or services, or have a financial interest in the companies whose materials were presented in this study.

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