



Effect of Remineralizing Agents on Tooth Color after Home Bleaching

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ABSTRACT

Objectives: The purpose was to investigate the effect of casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF) paste, Remin Pro paste, and 0.05% sodium fluoride (NaF) mouthwash on teeth discoloration after home bleaching.

Materials and Methods: In this in-vitro study, 48 intact and unstained human incisors were divided into four groups (n=12): control (Group 1), CPP-ACPF paste (Group 2), Remin Pro paste (Group 3), and 0.05% NaF mouthwash (Group 4). The specimens were bleached using 20% carbamide peroxide gel for 21 days and were subjected to pH cycling and surface treatment with remineralizing agents for one month. The color of the specimens was determined according to the CIELAB color space at baseline, after bleaching, and after surface treatment. Data were analyzed using one-way analysis of variance (ANOVA), post-hoc least significant difference (LSD) test, and one-sample t-test (P=0.05).

Results: ANOVA showed a significant difference in the mean ΔE of the four groups after surface treatment (P<0.05). Post-hoc LSD test showed a significant difference between the mean ΔE of the control group and those of the treatment groups (P<0.05) with no significant difference between the treatment groups, except for Remin Pro. The mean ΔE and ΔL of the Remin Pro group showed less change than those of the CPP-ACPF and NaF groups, indicating that Remin Pro has the lowest potential for post-bleaching tooth discoloration.

Conclusion: CPP-ACPF paste, Remin Pro paste, and 0.05% NaF mouthwash cause noticeable teeth discoloration immediately after bleaching. Remin Pro has less effect on tooth color than the other two products.

Keywords: Tooth Bleaching; Tooth Remineralization; Spectrophotometer

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INTRODUCTION

In the last few decades, people have become obsessed with having a more aesthetically pleasing smile, and thus, tooth color is one of their top concerns [1,2]. When performed with proper technique, tooth whitening can be a

conservative and safe approach to treat discolored teeth [3]. Home bleaching techniques have received worldwide acceptance after being described for the first time in 1989 by Haywood and Heymann [4,5]. It is now a common practice to whiten

discolored teeth using low concentrations of carbamide peroxide ($\text{CO}(\text{NH}_2)_2 \cdot \text{H}_2\text{O}_2$) and hydrogen peroxide (H_2O_2) [1,6]. Carbamide peroxide (6-35% w/v) is widely used as a tooth bleaching agent; it produces H_2O_2 and urea when comes in contact with water or saliva [7,8]. However, the low pH of some bleaching agents, byproducts of protein denaturation (e.g. urea), long exposure of tooth surface to bleaching agents, and different concentrations of bleaching agents can cause tooth demineralization and sensitivity [1,9-11]. To overcome mineral loss, several modified carbamide peroxide compounds have been developed. The most notable examples of these modifications are the addition of fluoride and calcium to the product [6] and the use of casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF) paste, also known as MI paste, with carbamide peroxide. Some studies have suggested that MI Tooth Mousse can be used concomitantly with a tooth whitening agent at a 1:1 ratio without impeding its bleaching effect [12-15].

Remin Pro paste is a newer water-based remineralizing cream containing hydroxylapatite, fluoride, and Xylitol, which can significantly increase the microhardness of dental structures when used after bleaching [16]. In this respect, Remin Pro is comparable to previous brands like GC Tooth Mousse. This increase in microhardness is due to 1540 parts-per-million (ppm) of fluoride contained in Remin Pro, which is 61% more than that of other brands (900 ppm in GC Tooth Mousse Plus) [16,17]. Dentists, however, are concerned about the post-treatment discoloration caused by such remineralizing agents, which could disappoint patients who prefer whiter teeth. There are several methods to assess the effectiveness of a bleaching agent, including the use of shade guides, photography, colorimeters, spectrophotometers, and computer digitization [18-20]. The CIELAB system is a particularly useful tool for quantifying the optical properties of teeth [21].

Bleached teeth have higher L^* values and lower b^* values but the a^* value appear to be

relatively unaffected [22-24]. In an attempt to establish which of the parameters could strongly influence the total color change (ΔE) of a tooth, few studies have found a linear correlation between each of the L^* , a^* , and b^* parameters and the color variations expressed as ΔE [25]. To the best of our knowledge, the effect of remineralizing agents on post-bleaching tooth discoloration has not been investigated. In view of this gap in the literature, the purpose of this study was to evaluate the effect of different remineralizing agents on teeth color after home bleaching and to discover the relationship between L^* , a^* , and b^* parameters and ΔE .

The null hypotheses of the study are:

- 1) The use of remineralizing agents after bleaching does not affect the color of the teeth.
- 2) There is a similar relationship between the L^* , a^* , and b^* parameters and ΔE .

MATERIALS AND METHODS

Test design:

The factors under study were:

- (1) Three different types of remineralizing agents, including CPP-ACPF paste, Remin Pro paste, and 0.05% NaF mouthwash.
- (2) Three different time points: baseline (T0), after bleaching (T1), and after the application of the remineralizing agent (T2).

The specimens consisted of 48 human incisors randomly assigned to four groups ($n=12$) and subjected to a remineralizing agent according to the manufacturers' recommendations.

Specimen preparation:

Forty-eight newly extracted, non-decayed, and unstained permanent central and lateral incisors were put in 0.2% saturated thymol solution and were placed in a refrigerator at 4°C for one week. Before the color evaluation test, the specimens were subjected to scaling and root planing (SRP), brushed and then placed in artificial saliva during the test process.

Baseline color (T0) assessment:

The color of the specimens was measured using a spectrophotometer (ShadePilot TM, DeguDent GmbH, Rodenbacher, Germany), and their L^* , a^* , and b^* values were determined. In this system, L^* represents the lightness and

ranges between 0 (dark) and 100 (bright), a^* represents the color's redness (+) versus its greenness (-), and b^* represents the color's yellowness (+) versus its blueness (-).

To standardize the area where color is measured, every tooth was coated with two layers of red lacquer (Shaima nail polish, Turkey), except for a 4×4-mm² window at the middle third of the labial surface [26].

Bleaching process:

The bleaching treatment was performed 8 hours per day for 21 consecutive days with 20% carbamide peroxide (Ultradent Products Inc., South Jordan, Utah, USA) according to the manufacturer's instructions. Every day, 0.04 ml of the bleaching agent (roughly a 1-mm-thick layer) was applied to the exposed enamel surface. The crowns of the teeth were covered with cellophane and secured with orthodontic rubber bands. The specimens were immersed in artificial saliva and were incubated at 37°C. The artificial saliva was replaced daily. After 8 hours of carbamide peroxide application, the gel was washed with distilled water for 30 seconds.

Second color (T1) assessment:

Immediately after bleaching, the color of the specimens was reevaluated using the spectrophotometer and according to the CIELAB system (L^* , a^* , and b^* parameters). The colors were compared using the ΔL^* , Δa^* , and Δb^* parameters as well as the total color difference (ΔE) as calculated by the following equation:

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

The lowest ΔE that can be differentiated by the human eye is 3.3 [27].

pH cycling and surface treatment:

The specimens were subjected to a pH cycling process for one month as follows [28]:

- A) Three hours of demineralization in demineralizing solutions including fluoride (0.2 ppm), lactic acid (0.05 mM), NaH_2PO_4 (2.2 mM), CaCl_2 (2.2 mM); the pH was adjusted at 4.5 using 50% NaOH.
- B) Washing with distilled water.
- C) Twenty-one hours of remineralization in artificial saliva with the following formulation: NaCl

(4.2 mM), Na_3PO_4 (3.9 mM), H_2SO_4 (0.5 mM), MgCl_2 (0.08 mM), CaCl_2 (1.1 mM), KCl (17.9 mM), and NaHCO_3 (3.2 mM), with the pH of 7.2.

- D) Surface treatment with different remineralizing agents.
- E) Re-washing with distilled water.

Three groups were used for the experiment, and one group was reserved as a control:

Group 1: Control group (immersion in artificial saliva with no treatment).

Group 2: Treatment with 1g of MI Plus (CCP-ACPF) paste (Tooth Mousse/MI Paste-TM/MI, GC Corp., Tokyo, Japan) for three minutes.

Group 3: Treatment with 1g of Remin Pro paste (VOCO GmbH, Cuxhaven, Germany) for five minutes.

Approximately a 1-mm-thick layer of the pastes was actively applied to the exposed enamel surface. After the recommended time, the pastes were washed with distilled water for 20 seconds.

Group 4: Immersion in 0.05% sodium fluoride (NaF) mouthwash (Epimax, Emad Pharmaceutical Co., Esfahan, Iran) for one minute. Each tooth was separately immersed in a plastic tube containing NaF mouthwash so that the exposure level was upward, and the teeth were in contact with the solution.

Final color (T2) assessment:

After surface treatment and pH cycling, the color of the specimens was reevaluated by the same method and the same formula as described above. The color assigned at T1 and T2 for each specimen was converted to the previously established numeric values. The color difference was determined by calculating the difference between T1 and T2 values. For CIELAB data, the colors were compared using ΔL^* , Δa^* , and Δb^* parameters as well as the total color difference (ΔE) according to the following formula:

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

Statistical analysis:

The discoloration data obtained using the CIELAB system were analyzed by one-way analysis of variance (ANOVA), post-hoc least significant difference (LSD) test, and one-sample t-test ($P=0.05$).

RESULTS

ΔE :

One-way ANOVA showed a significant increase in the mean ΔE of all groups after bleaching (Fig. 1). However, all four groups were whitened to the same extent, and there was no significant difference between them in this respect (Table 1).

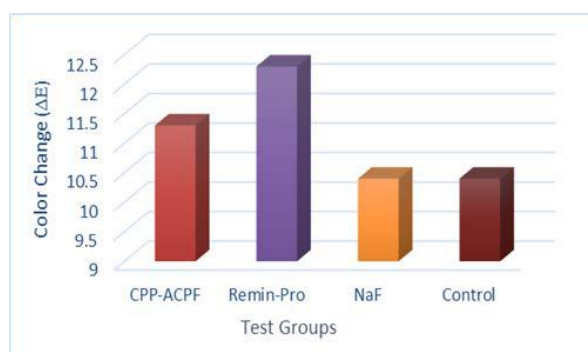


Fig. 1. ΔE values for test groups after the bleaching process

Table 1: The mean and standard deviation (SD) of ΔE for different materials after bleaching

Group	Color Change (ΔE)			
	Mean	SD	Min	Max
CPP-ACPF	11.3	0.56	10.60	11.96
Remin Pro	12.3	0.45	11.50	12.90
NaF	10.4	0.35	9.55	10.88
Control	10.4	0.45	9.50	11.10

CPP-ACPF: casein phosphopeptide amorphous calcium phosphate fluoride, NaF: sodium fluoride

A significant difference in the mean ΔE was observed after remineralization ($P < 0.05$; Table 2). Post-hoc LSD test showed a significant difference in the ΔE of the treatment groups and that of the control group ($P < 0.05$) but there was no significant difference between the three treatment groups, except for the Remin Pro group (Fig. 2).

According to this analysis, the greatest changes in ΔE were caused by NaF, CPP-ACPF and Remin Pro, respectively, but one-way ANOVA found no significant difference between the treatment groups in terms of the mean change in ΔE . One-sample t-test showed

Table 2: The mean and standard deviation (SD) of ΔE for different materials after remineralization

Group	Color Change (ΔE)		Min	Max
	Mean	SD		
CPP-ACPF	7.11	0.31	6.25	7.55
Remin Pro	6.68	0.35	6.20	7.15
NaF	7.29	0.28	6.70	7.80
Control	3.66	0.14	3.50	3.90

CPP-ACPF: casein phosphopeptide amorphous calcium phosphate fluoride, NaF: sodium fluoride

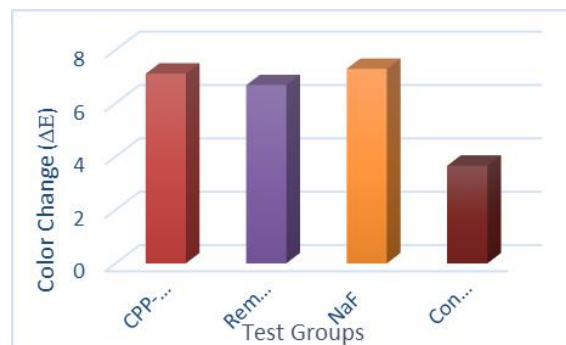


Fig. 2. ΔE values for test groups after remineralization

that in all three treatment groups, the mean ΔE_{T0-T1} and ΔE_{T1-T2} were greater than the maximum value (3.3) acceptable in the visual assessment of the color in dentistry.

ΔL :

For all groups, ANOVA showed the statistical significance of the mean ΔL_{T1-T2} ($P < 0.05$). The results of the LSD test showed that the mean ΔL values in the CPP-ACPF and NaF groups were not significantly different and that both agents increased the ΔL to the same extent ($P = 0.4$), which was greater than that of the Remin Pro group (Table 3).

Δa :

For all groups, ANOVA showed a statistically significant difference of the means Δa_{T1-T2} ($P < 0.05$). In both CPP-ACPF and NaF groups, Δa values were found to be less than zero ($\Delta a < 0$), which indicates a tendency towards green color. The highest Δa was observed in the NaF group. For the control and Remin Pro groups, this value was more than zero ($\Delta a > 0$), which indicates a tendency towards red color (Table 3).

Table 3: The mean and standard deviation (SD) of ΔE , ΔL , Δa , and Δb for different materials after remineralization

Group	Color Change (ΔE)	Whiteness (ΔL)	Yellowness (Δa)	Redness (Δb)
CPP-ACPF	7.11±0.31	4.82±0.36	-0.6±0.42	0.52±0.25
Remin Pro	6.68±0.35	2.56±0.38	0.43±0.40	1.39±0.28
NaF	7.29±0.28	4.72±0.28	-3.8±0.29	-0.31±0.32
Control	3.66±0.14	0.02±0.20	0.67±0.09	±0.11 2.92

CPP-ACPF: casein phosphopeptide amorphous calcium phosphate fluoride, NaF: sodium fluoride

 Δb :

ANOVA showed a statistically significant difference in the mean Δb_{T1-T2} ($P < 0.05$).

In all groups, except for NaF, Δb values were greater than zero ($\Delta b > 0$). The highest Δb value was observed for the control group, which signifies the high tendency towards yellow color (Table 3).

DISCUSSION

Tooth bleaching is a conservative and safe approach to treat discolored teeth [3]. Home bleaching is a globally accepted technique in this field of treatment [4].

Demineralization of dental structures is one of the important post-bleaching effects caused by the low pH of some bleaching agents and dental sensitivity; this compels dentists to prescribe remineralizing products [9-11].

About two-thirds of the patients undergoing this treatment experience some degrees of transient dental sensitivity during and after bleaching [29].

Oxide-reduction reaction of the bleaching agent can lead to solving of organic and mineral matrices to the extent that only carbon dioxide (CO_2) and water remain [30]. Although saliva can induce post-bleaching enamel remineralization in vitro [31] and in vivo [32], studies have shown some degrees of microhardness reduction in bleached enamel.

Many have suggested the use of remineralizing agents, such as fluoride, to overcome the problems caused by demineralization; however, the results in this regard have been inconsistent. Burgmaier et al [33] found no significant increase in fluoride uptake during enamel bleaching but Borges et al [34] have stated that the use of fluoride after bleaching improves enamel microhardness and prevents enamel demineralization. Attia and Kamel

[35] have indicated that GC Tooth Mousse Plus and Remin Pro are more effective in reducing enamel surface roughness after bleaching compared to fluoride-containing products. Furthermore, research on different formulations of peroxide with calcium and fluoride has shown that the use of fluoride and/or calcium prevents microhardness reduction and accelerates the recovery of post-treatment microhardness to the pre-treatment state [6]. The ACP that is stabilized by CPP can provide a higher reservoir of calcium and phosphate ions than the ACP alone; this increases the microhardness and remineralization potential of CPP-ACP. However, CPP-ACP also has other merits; for example, it is less likely to cause clinical dental hypersensitivity [36]. It has been shown that, when compared to NaF-containing fluoride gels, ACP-containing gels have a greater effect on the remineralization of predemineralized bovine teeth [37].

In-vivo studies have shown that the use of MI paste significantly decreases dental sensitivity without compromising the whitening properties of bleaching agents [20]. The purpose of the application of MI paste after bleaching is to reduce dental sensitivity and reverse the demineralizing effect of low-pH solutions; it also improves aesthetics by increasing luster and translucency [25].

Despite its benefits, the use of this commercial solution after bleaching has the potential to cause dental discoloration, which may disappoint the patients who desire whiter and more lustrous teeth. Studies have shown that the use of 2% NaF does not interfere with the bleaching effect of 35% H_2O_2 [38]. These results are consistent with the evidence provided by Armênio et al [39] (2008) who examined the effect of 1.23% fluoride gel applied for four minutes after daily use of 16%

carbamide peroxide gel. The cited study indicated no significant difference in bleaching results of the treatment group and the control group at the end of the bleaching process; this shows that NaF does not undermine the whitening effect of peroxide gel [39]. Chen et al [40] (2008) have reported that fluoridated bleaching agents and fluoride treatments after bleaching do not interfere with bleaching effects. Also, Maran et al [41] (2018) have reported that the incorporation of NaF in 10% carbamide peroxide at-home bleaching gel does not affect the color change. These reports are in contradiction with our findings that showed the highest ΔE after the use of NaF compared to other solutions.

Kim et al [42] (2011) have reported that NaF can produce visible discoloration after bleaching ($\Delta E=7.51$), which is consistent with our results ($\Delta E=7.29$). In addition, it has been reported that CPP-ACP (MI) paste can prevent the undesirable effects of bleaching on enamel roughness and hardness without affecting the bleaching ability [12-15]. However, our results challenge this report as we found that CPP-ACPF (MI Plus) paste can cause visible discoloration in bleached teeth ($\Delta E=7.11$). This is probably due to the addition of fluoride to MI Plus paste compared to previous products.

Remin Pro has been marketed as a water-based remineralizing cream, which can significantly increase the microhardness of dental structures when used after bleaching [15]. To the best of our knowledge, the present study is the first investigation on the discoloration potential of Remin Pro; therefore, there is no reference for comparison of the results with regard to this product.

The color data were gathered using a spectrophotometer and were evaluated with the three coordinate values (L^* , a^* , and b^*) established by the CIE, which is widely accepted in dental bleaching research as a valid index for identification of color using a three-dimensional (3D) color space. In this system, discoloration is expressed by ΔE , and ΔE values of more than 3.3 are visually noticeable [22].

In the present study, the range of post-bleaching ΔE was between 10.4 and 12.3, and all the tested bleaching protocols showed very noticeable whitening effects. Changes in ΔE after remineralization treatment were between 6.68 and 7.29, which are also visually noticeable.

Some studies have reported that discoloration is mostly a function of b^* and L^* parameters as they have higher mean values and are inversely correlated; the least significant potential of change can be expected with the a^* parameter [22-24,43]. Despite such reports, no linear correlation tests have been proposed to verify the actual influence of each parameter on ΔE .

The results of this study indicate that the a^* parameter has an inverse correlation with ΔE and affects the discoloration to the same extent as L^* and b^* parameters. The minimum ΔE was observed in the Remin Pro group ($\Delta E=6.68$), which indicates that Remin Pro has the lowest potential for post-bleaching tooth discoloration compared to other remineralizing agents.

CONCLUSION

All groups exhibited a similar effect on bleaching performance in the form of an increase in whiteness and a decrease in yellow color saturation. NaF solution showed a noticeable dental discoloration potential and is therefore not recommended as a remineralizing agent. Remin Pro exhibited the least post-bleaching discoloration potential and is more suitable than alternative solutions.

CONFLICT OF INTEREST STATEMENT

None declared.

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