Original Article

In-Vitro Effect of Casein Phosphopeptide Amorphous Calcium Phosphate on Enamel Susceptibility to Staining by Tea during Bleaching Treatment

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Abstract:

Objectives: Bleached enamel is more susceptible to staining, and application of remineralizing agents may decrease enamel susceptibility to staining. This study sought to assess the effect of casein phosphopeptide amorphous calcium phosphate (CPP-ACP) on enamel susceptibility to staining during bleaching treatment.

Materials and Methods: Forty central and lateral incisors and first premolar teeth were evaluated in four groups of 10. Group one specimens were subjected to in-office bleaching. Group two underwent in-office bleaching followed by surface treatment with CPP-ACP. Group three specimens received home bleaching and group four underwent home bleaching followed by CPP-ACP surface treatment. After each course of daily bleaching, specimens were immersed in tea solution. Home bleaching (15% carbamide peroxide) was performed for 14 days and in-office bleaching (40% hydrogen peroxide) was carried out in two sessions with an eight-day interval. The color of specimens was analyzed at baseline and post-intervention using EasyShade Shade-Selection Device. Two-way ANOVA was used to evaluate the effects of bleaching type and surface treatment on color change. Then, the means were compared by Tukey's HSD test (P=0.05).

Results: The interaction effect of surface treatment and type of bleaching was not significant on any color parameter (P>0.05). Surface treatment had significant effects on ΔL (P=0.004). Type of bleaching had a significant effect on "b" parameter (P=0.00). The effect of bleaching type on ΔE was significant (P=0.00) but the effect of surface treatment was not (P=0.34).

Conclusion: CPP-ACP had no significant effect on preventing enamel staining by tea during bleaching treatment.

Keywords: Casein Phosphopeptide-Amorphous Calcium Phosphate Nanocomplex; Tooth Bleaching; Staining; Tea

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INTRODUCTION

Vital and non-vital tooth bleaching is an important part of operative dentistry. It is a treatment option for discolored teeth without the need for using tooth-colored restorative materials [1].

Tooth bleaching is a safe, effective and minimally invasive treatment modality for tooth discolorations. Two techniques are commonly used for vital tooth bleaching: in-office bleaching and home bleaching [2]. Although macroscopically no clinical harm is

done to bleached teeth, some scientific evidence reports changes in histology and mineral content of bleached enamel. These changes include decreased microhardness and increased porosity and permeability [2-7]. Changes in bleached enamel may be due to the oxidizing and demineralizing effects of hydrogen peroxide [5]. The commonly used bleaching agents contain hydrogen peroxide that can be converted into hydroxyl radicals when in contact with teeth. Due to low molecular weight, hydroxyl radicals quickly and easily penetrate into the enamel and dentin porosities and cleave the weak bonds between stain molecules and organic matrix into smaller, less complex molecules [8]. Carbamide peroxide, commonly used in home bleaching products, breaks down into hydrogen peroxide and urea in presence of water or saliva. Hydrogen peroxide and urea further break down into water, oxygen, carbon dioxide and ammonia. This process is associated with a drop in pH of the bleaching agent causing the dissolution of the enamel mineral content [9].

Foods and drinks (like tea) consumed after the bleaching treatment (either immediately or after a while) can stain the bleached enamel. Several studies have investigated staining of teeth following bleaching treatments. In the majority of these studies, the bleached enamel showed higher susceptibility to staining and the treatment results were not stable [1,5,10-15]. Also, the susceptibility of teeth bleached with 35% hydrogen peroxide was greater than that of teeth bleached with 16% carbamide peroxide [10]. Such color instability and increased stainability may be due to the microscopic changes that occur in the enamel surface including increased micro-roughness, enamel porosities and permeability of enamel after bleaching treatment [4-6,10]. Remineralization of demineralized enamel is a gradual process that may occur partially or incompletely; thus, application of agents that accelerate this process is recommended following tooth bleaching [4].

It is assumed that surface treatment of bleached enamel decreases post-operative tooth hypersensitivity and enamel stainability and increases the durability of bleaching treatment [16,17]. It has been shown that CPP-ACP nanocomplexes can decrease demineralization and enhance the remineralization of carious lesions under in-vitro and in-vivo conditions [18]. The GC Tooth Mousse is a commercial product with CPP-ACP as its main constituent. Casein phosphopeptide is derived from milk protein and is significantly capable of stabilizing calcium phosphate in dental plaque [18]. Amorphous calcium phosphate can obstruct dentinal tubules by quick deposition of calcium and phosphate ions on the surface and inside the tubules [8]. The CPP is an active sequence of amino acids and is significantly capable of stabilizing calcium and phosphate as nanoclusters of ions in a semi-stable solution [19]. The CPP binds to calcium and phosphate in nanoparticles and prevents their growth to the critical size and deposition; by doing so, it stabilizes calcium and phosphate ions in a solution [20]. It has been reported that CPP-ACP can increase the microhardness of acid-eroded as well as bleached enamel [18,21]. Considering the fact that the aforementioned enamel changes are responsible for its susceptibility to staining, using CPP-ACP may provide optimal results in terms of stability of treatment outcome and reducing tooth hypersensitivity. Application of this agent may also help remove dietary restrictions that are usually recommended for patients receiving bleaching treatments. The ability of commonly consumed drinks like tea to cause staining has reported to be variable in different studies [12,16]. Moreover, number of studies comparing the susceptibility of teeth to staining following in-office and home bleaching treatments is scarce [10]. Thus, this study was designed to assess the effect of CPP-ACP (GC Tooth Mousse) on decreasing enamel susceptibility to staining by tea during two different methods of bleaching treatment.

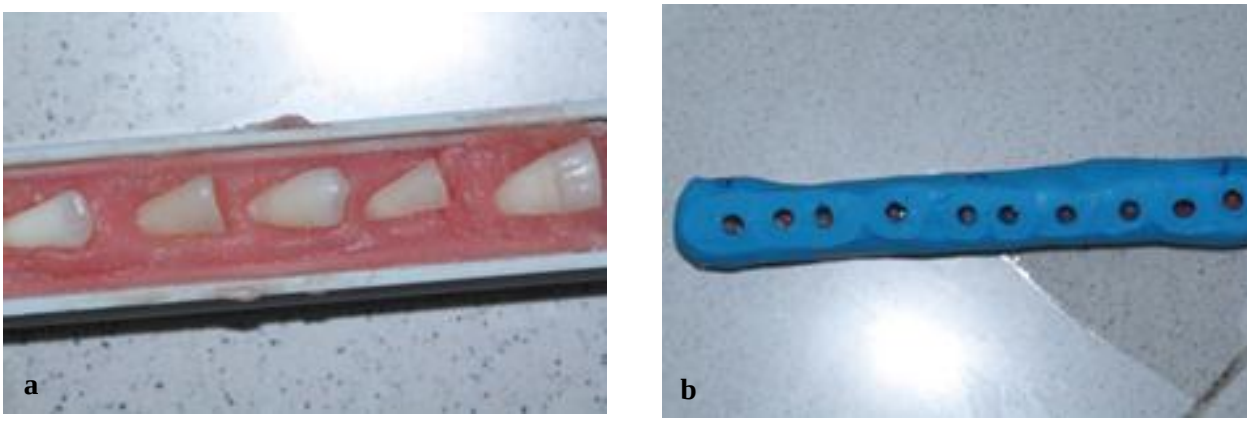

Fig. 1. Mounting of specimens (a) and custom silicon impression (b)

The null hypothesis tested was that GC Tooth Mousse would have no significant effect on decreasing enamel susceptibility to staining by tea during bleaching treatment and that the results of in-office bleaching and home bleaching would not be significantly different.

MATERIALS AND METHODS

Preparation of specimens:

Forty central and lateral incisors and first premolar teeth with no cracks, caries or significant discoloration extracted for orthodontic or periodontal purposes were collected and stored in saline solution for a maximum of four months. The teeth were cleaned from debris, calculus and superficial stains using Cavitron, prophylactic paste and rubber cup. Two weeks prior to the experiment, the teeth were immersed in artificial saliva (Biotene, Laclede Inc., CA, USA). Specimens were divided into four groups of 10 each and mounted in autopolymerizing acrylic resin in such a way that only the labial surface of the teeth was out of the acrylic resin. The experimental groups were as follows:

1-Office bleaching + surface treatment (CPP-ACP)

2-Office bleaching

3-Home bleaching + surface treatment (CPP-ACP)

4-Home bleaching

The artificial saliva was refreshed daily and the specimens were kept in an incubator at 37°C for the time intervals between the bleaching and surface treatments. Moreover, the specimens were cleaned daily with 10 vertical motions of a toothbrush (Oral-B, Weybridge, UK) with toothpaste (Crest Complete 7, Weybridge, UK).

Colorimetery:

After cleaning the teeth and prior to the bleaching treatment, all specimens underwent color analysis using a spectrophotometer (Vita Easy Shade, Vivadent, Brea, CA, USA) and their color was analyzed and recorded using the CIE-Lab system. To ensure the reproducibility of color analysis and that the color of each specimen would be assessed again at the exact same point following the intervention, a silicone impression was made from the stylus of the device and poured with acrylic resin.

Ten acrylic resin models were fabricated as such and fixed on the middle third of each tooth surface. A silicone index was then made for each group in such a way that it embraced the acrylic. By doing so, we ensured the reproducibility of the position of the spectrophotometer stylus relative to the specimen surface (Fig. 1).

Bleaching and surface treatments:

Groups three and four specimens (in-office bleaching) were subjected to 40% hydrogen peroxide bleaching agent (Opalescence,

Ultradent Products Inc., South Jordan, UT, USA) for 20 minutes for a total of three times according to the manufacturer's instructions. The bleaching treatment was repeated one week later. Group three specimens received surface treatment with CPP-ACP for five minutes daily during the time interval between the two bleaching treatments and were then cleaned by gauze according to the manufacturer's instructions. The specimens were stored in artificial saliva for one hour followed by immersion in tea solution (Lipton Yellow Label Tea, London, UK) for 10 minutes. Next, the specimens were washed with water and stored in artificial saliva until the next day. In groups one and two (home bleaching), 15% carbamide peroxide (Opalescence Ultradent Products Inc., South Jordan, UT, USA) was applied to the surface of specimens in one millimeter thickness for six hours daily according to the manufacturer's instructions. Surface treatment (group one) and immersion in tea solution were carried out as described earlier. For the preparation of tea solution, a tea bag was placed in 250 mL of boiling distilled water for three minutes. It should be noted that the specimens were immersed in tea solution in an incubator at 55°C. Materials and their compositions are shown in Table 1. After the completion of home and in-office bleaching treatments, the specimens were immersed in artificial saliva for rehydration and then underwent color analysis by spectrophotometer (Vita Easy Shade).

Finally, ΔE of specimens was calculated using the formula below:

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

Statistical analysis:

Data were analyzed using SPSS version 20. The results were analyzed using, two-way ANOVA. The mean values between the groups were analyzed using the Tukey's HSD test with alpha set at 0.05.

RESULTS

According to ANOVA, the interaction effect of surface treatment and type of bleaching treatment was not significant on "L" (P=0.66), "a" (P=0.19), "b" (P=0.56) or "E" (P=0.53).

The mean and standard deviation of changes in "L", "a", "b" and "E" parameters are shown in Table 2.

The maximum ΔE was seen in group three (home bleaching with surface treatment) and the minimum ΔE was seen in group two (in-office bleaching).Since the effect of bleaching type on ΔL values was not significant (P=0.55), surface treatment had a significant effect on this parameter, and groups with surface treatment (one and three) had greater color change (P=0.004). Neither the type of bleaching (P=0.16) nor the surface treatment (P=0.71) had significant effects on Δa.

Type of bleaching had a significant effect on changes of "b" parameter and groups three and four (home bleaching) showed higher Δb values (P=0.00). Bleaching type also had a significant effect on ΔE values (P=0.00), but the effect of surface treatment was not significant in this regard (P=0.34).

DISCUSSION

Color is a psychological subjective phenomenon and different individuals may have different perceptions of the same color.

Table 1. Study materials and their composition

Material	Type of material	Manufacturer	Composition
Opalescence 15%	Bleaching gel	Ultradent USA	Carbamide peroxide, potassium nitrate 0.11% fluoride ion
Opalescence 40%	Bleaching gel	Ultradent USA	Hydrogen peroxide
GC Tooth Mousse	Remineralizing paste	GC Japan	-3% potassium nitrate-1.1%flouride

Color analysis with meticulous tools eliminates the subjective errors [22]. In this study, the color of specimens was analyzed using EasyShade Shade-Selection Device. It uses a spectrophotometer and is capable of quantitative measurement of color changes. Previous studies have confirmed the high accuracy and reliability of this device [23]. To simulate the clinical setting, specimens were stored in artificial saliva at 37°C and the tea solution had a temperature of 55°C.

Our study showed that all specimens had ΔE>3.3 after bleaching treatment. This value is considered as the threshold of color change detection by the untrained eye [22, 24]. During the bleaching treatment, the specimens were immersed in tea solution. All specimens had ΔE>3.3, which indicates the acceptable and significant effects of bleaching. Based on our results, we cannot conclude that tea did not affect the results of bleaching treatment but we may state that despite the staining effect of tea, both bleaching treatments had acceptable efficacy. Attin et al, in their study reported that daily application of tea solution did not significantly affect the results of bleaching treatment [12]. However, Singh et al. demonstrated that tea had a significant effect on the color change of freshly bleached enamel and this effect was manifested by the shift of "ΔL" parameter towards the negative (darker) direction [16]. Specimens exposed to tea solution in their study showed ΔE<3.3 at one hour after bleaching treatment; which means that the bleaching results in their study were lower than the clinically acceptable color change.

Such difference between our results and those of Singh et al. may be due to the fact that in their study the teeth were bleached with 10% carbamide peroxide for a period of eight days; whereas, we used 15% carbamide peroxide for 14 days (home bleaching). Moreover, in our study, the teeth were brushed with 10 vertical motions of a toothbrush along with toothpaste to remove superficial stains. In the study by Attin et al, [12] the concentration of bleaching agent and duration of treatment were similar to those of Singh et al [16]; however, in the study by Attin et al, the specimens were polished after bleaching and tea staining and then underwent color analysis [12]. Total color change in our study groups is attributed to the shift of "L" parameter towards the positive and shift of "a" and "b" parameters towards the negative direction. In other words, the "L" parameter shifted towards lighter shades, "a" shifted towards less redness and "b" shifted towards less yellowness; which are all in accord with the results of Attin et al, [12] Russo et al, [14] and Wiegand et al [1]. The "L" and "b" parameters had the greatest impact on ΔE; which is in agreement with the previous studies [1,3, 16]. Our results demonstrated that "ΔE" of home bleached groups was significantly greater than that of office bleached specimens. In a systematic review in 2011, it was concluded that in-office bleaching was more efficient in the first week of treatment than home bleaching but the outcome of both techniques was similar in the second week post-treatment [25]. Since in our study, the duration of bleaching treatment was two weeks, the lower efficacy of in-office bleaching may be explained by the

Table 2. The mean and standard deviation of changes in "L", "a", "b" and "E" parameters

	ΔL	**Δa**	**Δb**	**ΔE**
Office bleaching+ CPP-ACP	4.38(2.25)	-.86(0.85)	-1.73(2.36)	5.27(2.42)
Office bleaching	2.07(2.80)	-1.23(1.69)	-2.99(2.76)	4.98(2.65)
Home bleaching + CPP-ACP	5.30(3.40)	-1.93(1.28)	-7.18(2.54)	9.67(2.90)
Home	2.21(2.58)	-1.27(0.94)	-7.45(3.11)	8.26(3.21)

higher stain absorption of in-office bleached specimens due to the use of higher concentration of hydrogen peroxide compared to home bleaching; this finding confirms the results of Setien et al [10]. Surface treatment with CPP-ACP in our study had no significant impact on "ΔE" of specimens. Kim et al. reported that CPP-ACP had no significant effect on color stability of bleached teeth [26]. Publio et al. demonstrated that CPP-ACP had no significant influence on enamel susceptibility to staining by cigarette smoke following bleaching treatment and reported that the remineralizing effect of saliva was greater than that of CPP-ACP [17]. However, Singh et al. indicated that CPP-ACP prevented the staining of freshly bleached enamel by tea solution [16]. It should be noted that in our study, the effect of CPP-ACP on "L" parameter, which indicates lightness, was significant and the groups that received surface treatment had higher "ΔL" values than those without surface treatment (Table 2). Our results in this respect were similar to those of Singh et al [16]. However, in our study, the effect of CPP-ACP on "b" parameter was not significant. These results may indicate that CPP-ACP can maintain the lightness achieved by bleaching treatment but cannot prevent the absorption of yellow tea stains into the bleached enamel and consequently, no significant change occurs in ΔE of specimens.

In the study by Singh et al, "Δb" was not significantly different between groups that received surface treatment and those that did not. They described that changes in "ΔE" were attributed to changes that occurred in "ΔL" [16]. Based on the literature, the "b" parameter is the most important indicator of bleaching efficacy [1]. Thus, absence of a significant effect by CPP-ACP surface treatment on this parameter can explain its ineffectiveness on the bleaching outcome. Remineralizing agents like CPP-ACP remineralize the tooth structure and decrease the porosities caused by the bleaching treatment [17, 20]. This property may decrease stain absorption into the tooth structure. On the other hand, remineralizing agents obstruct the dentinal tubules [8] and may consequently decrease the efficacy of bleaching treatment. It appears that the interaction of these two effects is responsible for its small or no impact on the results of bleaching treatment.

CONCLUSION

Within the limitations of this study, it was concluded that CPP-ACP had no significant effect on prevention of tea stain absorption into enamel during the bleaching treatment.

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