Effect of CPP-ACP and sodium fluoride on prevention of re-staining after bleaching

Yumi Imamura, DDS, Masayuki Otsuki, DDS, PhD, Alireza Sadr, DDS, PhD, and Junji Tagami DDS, PhD

Cariology and Operative Dentistry, Department of Oral Health Sciences, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University. Tokyo, Japan

Purpose: This study evaluated the effect of sodium fluoride (NaF) solution and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) paste on the prevention of re-staining after in-office bleaching *in vitro* using an artificial discoloration tooth model.
Materials and Methods: Fifty extracted bovine teeth were stained in black tea and bleached by an in-office beaching material (TiON In Office). The teeth were divided into five groups, with 10 teeth in each group (n=10). They were treated with 2.0% or 0.2% NaF solution, CPP-ACP paste (MI Paste) or CPP-ACP paste containing fluoride (MI Paste Plus) for 1 hour. A group left without application served as control group. Then, each tooth was immersed into the black tea again. CIE L*a*b* of the stained surface of each tooth was measured by a dental color meter (Shade Eye NCC) after bleaching, at 1, 3, and 6 hours, and 1, 2, 3, and 7 days after immersion, and the changes in color were evaluated. Other specimens were prepared and the bleached and stained surfaces were observed by a scanning electron microscope (SEM) at each period.
Results: Color change increased gradually by immersion in the black tea in all groups. The color change in all experimental groups was less than that of control group. Repeated measure ANOVA showed that both the treatment solution and the immersion time significantly affected ΔE ($p<0.05$). A remarkable difference was not found in the SEM images among all groups at each period.
Conclusion: It was concluded that application of NaF and CPP-ACP would be able to decrease re-staining and discoloration of teeth after office-bleaching. **(Asian Pac J Dent 2013; 13: 47-55.)**
Key Words: CPP-ACP, re-staining, sodium fluoride, tooth bleaching

Introduction

The tooth bleaching has become one of the most popular esthetic dental treatments, which is rather conservative and cost-effective for improving or enhancing a person's smile.[1] The vital-tooth bleaching has been developed under two categories; in-office bleaching as professionally applied in the dental office and home bleaching as prescribed by the dentist for home-use by the patient. Current tooth bleaching materials are based primarily on either hydrogen peroxide or carbamide peroxide as active ingredients.[1]

Previous studies have shown a high patients' satisfaction with both office bleaching[2] and home bleaching.[3-5] However, tooth bleaching is not risk-free,[1] and safety issues have been raised regarding the effects of bleaching on the tooth structure,[6] pulp tissues,[7,8] and the mucosal tissues of the mouth,[9] as well as systemic ingestion.[1] It is known that at concentrations of 10% H_2O_2 or higher, this chemical substance is potentially corrosive to mucous membranes or skin, causing a burning sensation and tissue damage.[6,9,10] Severe mucosal damage can occur if gingival protection is inadequate.[1] Tooth sensitivity was also reported in various degrees and frequency after office bleaching and home bleaching.[11]

The tooth color relapse seems to be one of the biggest problems after bleaching. Such color relapse has been reported in both office bleaching[12] and home bleaching,[5,12] and the degree of color relapse has been various among the clinical researches.[5,12] In this regard, it is important that the patient understands that bleaching is not a permanent treatment and some periodic re-bleaching will be required, that can range from a few weeks to several months.[13] Post-bleaching color regression is a concomitant phenomenon in the vital teeth bleaching procedure; however, the phenomenon has not been well-understood.[14] It is therefore necessary to establish

preventive measures against re-staining after tooth bleaching.

Application of a solution containing active ingredients such as potassium nitrate,[2] sodium fluoride (NaF)[15] or casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) paste[16] has been recommended to prevent sensitivity after bleaching. NaF has been well known for its role for prevention of demineralization[17] and re-mineralization of enamel.[17,18] CPP-ACP also contributes for re-mineralization of decalcified enamel,[19] and may prevent demineralization.[20] Apart from sensitivity, both NaF and CPP-ACP have been suggested for the prevention of re-staining after tooth bleaching;[16] an interesting effect that desires further exploration.

The purpose of this study was to evaluate the effect of NaF solution and CPP-ACP pastes on the prevention of re-staining after office bleaching using the artificial discolored bovine tooth model *in vitro*. The null hypothesis was that the application of NaF or CPP-ACP pastes after bleaching did not affect re-staining of the tooth.

Materials and Methods

Specimen and initial staining

Fifty freshly extracted bovine incisors were prepared and artificially discolored according to previous studies.[21,22] The experimental procedure is shown in Fig. 1. The teeth were stored frozen after extraction and were thawed by running tap water at room temperature. The teeth were then cleaned by removing soft tissue remnants using a scalpel. The roots were cut and removed using a diamond disc (Separatedisk, Shofu, Kyoto, Japan) with a straight-type micromotor handpiece. Then, the pulpal tissue was removed by an endodontic file. The pulp chamber was irrigated with 5 mL of 5% sodium hypochlorite (Wako Pure Chemical, Osaka, Japan) to remove any tissue remnants followed by thorough washing, drying, and finally etching with 40% phosphoric acid gel (K-etchant, Kuraray Noritake Dental, Tokyo, Japan) for 10 s, to expose the tubule system and thereby increase stain uptake into the dentin.[23] Labial surfaces were ground to leave enamel of approximately 1 mm in thickness, and polished to create a smooth and flat enamel surface with ascending-grit silicon carbide papers (Sankyorikagaku, Saitama, Japan) starting from #280 up to #1,500 under running water. A stain solution was prepared by immersion of one black tea bag (2 g) (Nittoh-tea, Mitsui Norin, Tokyo, Japan) in 100 mL of boiled water for 5 minutes. Specimens were immersed in the solution and stored inside the incubator for 7 days at 37˚C. The solution was stirred once every day to avoid decantation of the solution and changed after every 3 days.

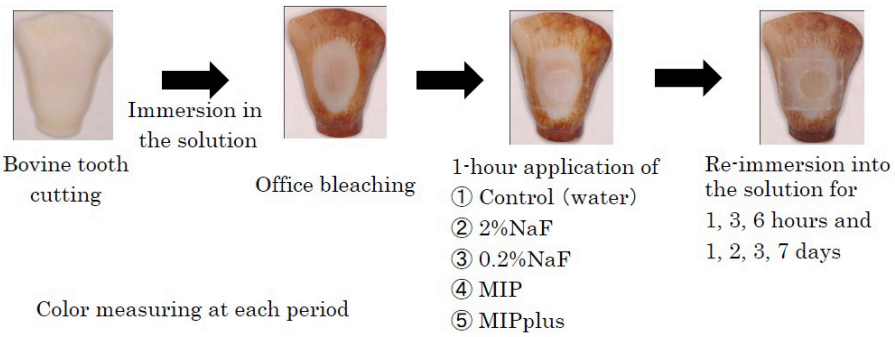

Fig. 1. Experimental procedure

Color measurement

After removing from the solution, the specimens were rinsed and dried. The labial surfaces were then covered with a masking tape with a 5 mm-diameter hole to fit the probe of a dental chroma meter (ShadeEye NCC, Shofu). This procedure ensured measuring the same area at each step. The CIE L*a*b* values of tooth surfaces

were recorded prior to bleaching for the first time as the baseline data, using the dental chroma meter and then the photograph of each experimental surface was taken by a digital camera. In order to decrease the variation among the specimens, only those specimens showing an L* value between 45 and 65 were included in the design. In this manner, a total of 50 teeth were selected for following experiment.

Tooth bleaching

The exposed canal of each specimen was sealed with an adhesive (Clearfil SE Bond, Kuralay Noritake Dental) and a composite resin (Esthelite Sigma Quick, Tokuyama Dental, Tokyo, Japan). The specimens were subsequently assigned into five groups (n=10), and treated with an office bleaching agent (TiON In Office, GC, Tokyo, Japan) according to the manufacturer's instructions as follows. The reactor was applied using a disposable brush, and its excess was removed by gentle air. Then the mixed bleaching gel was applied on the experimental surface and photo-irradiated for 12 minutes using a light unit (Cosmo Blue, GC), which is arch-type violet LED (λ=405 nm) lights for tooth bleaching. The bleaching gel was then wiped off from the experimental surface using a piece of damp gauze. The bleaching procedure was repeated three times. Finally, the specimen was washed thoroughly under tap water, dried gently and the color values of L*a*b* were measured using the chroma meter.

Application of NaF or CPP-ACP

In control group, teeth were stored in a container keeping 100% relative humidity for 1 hour without any surface treatment. For the experimental groups, the NaF solutions with different concentration (2.0% and 0.2%) were prepared and two types of CPP-ACP pastes were used in this study. In the first two groups, each bleached tooth was immersed in the 50 mL of 2.0% (2.0% NaF) or 0.2% (0.2% NaF) NaF solution for 1 hour. In groups 3 and 4, 0.2 g of either the CPP-ACP paste (MIP) or the CPP-ACP containing NaF paste (MIPplus) was placed on the bleached surface of each experimental tooth for 1 hour. Finally, all the specimens were washed with running tap water, dried gently and the color values of L*a*b* were measured using the chroma meter to serve as the baseline. The materials (bleaching and surface treatment agents) used in this study and their ingredients are presented in Table 1.

Table 1. Components and their ingredients

Materials	Component
TiON In Office (GC)	Reactor: Ethanol 80-90%, Water 10%, Titanium dioxide 1-5% Syringe A: Hydrogen peroxide aqueous solution 30-35% Syringe B: Carbamide peroxide (Urea hydrogen peroxide) 30-35%, Propylene glycol 60-65%
NaF (Wako Pure Chemical)	Sodium fluoride
MI Paste (GC)	Glycerol 10-20%, CPP-ACP 5-10%, *d*-Sorbitol 0-5%, Propylene glycol 0-2%, Silicon dioxide 0-2%, Titanium dioxide 0-2%, Ethyl-4-hydroxybenzoate <0.1%, Butyl-*p*-hydroxybenzoate <0.1%, Propyl-4-hydroxybenzoate <0.1%
MI Paste Plus (GC)	Glycerol 10-20%, CPP-ACP 5-10%, *d*-Glucitol 0-5%, Propylene glycol 0-2%, Sodium fluoride 0.2%, Silicon dioxide 0-2%, Titanium dioxide 0-2%, Ethyl-4-hydroxybenzoate <0.1%, Butyl-*p*-hydroxybenzoate <0.1%, Propyl-4-hydroxybenzoate <0.1%

Re-staining

The specimens in all groups were re-immersed in black tea at 37°C for 7 days, in the same manner as explained for the initial staining. The color of the specimen surface was measured at 1, 3, and 6 hours and 1, 2, 3, and 7 days after immersion. The difference of L*, a* and b* between the baseline and each period of the re-staining were expressed as ΔL, Δa, and Δb respectively. The color difference (ΔE) was calculated from the

values obtained at the baseline and after each period, according to the following equation.

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

Scanning electron microscope (SEM) observation

Additional specimens were prepared and subjected to SEM observation at various experimental stages; after initial staining, after bleaching, after treatment in each group, and after 7 days re-staining in each group. For this purpose, the specimens were dehydrated in a glass desiccator for 24 hours, sputter coated by gold and observed by SEM (JSM-5310LV, JEOL, Tokyo, Japan).

Statistical analysis

ΔE data of all groups was subjected to statistical analysis with repeated measures ANOVA test, with re-staining time as within subject factor and treatment group as between subject factor. Bonferroni correction was used as post hoc test for multiple comparisons. The statistical significance level was set as α=0.05.

Results

Digital photographs showing the typical color changes of the specimens in each group are presented in Fig. 2, and the average L*a*b* values of each period are demonstrated in Fig. 3. After the bleaching procedure, generally L* value was increased and a* and b* values were decreased and the bleaching effect was obvious. The calculated ΔE values were shown in Fig. 4; through the re-staining by the black tea extract, ΔE values were gradually increased in all groups accompanied by a decrease in the L* value. Generally, the color changes in any experimental groups were less than that of the control group. Repeated measures ANOVA showed that re-staining immersion time was a significant factor for all groups. Multiple comparisons suggested statistical differences between groups at all times beyond 1-hour re-immersion ($p<0.05$). After 1, 3, and 6 hours, and 1, 2, and 3 days of re-staining, ΔE values of all experimental groups were significantly different with that of the control group ($p<0.05$). After 7 days, 0.2% NaF, MIP, and MIPplus showed a statistically significant difference in ΔE value compared with the control group and 2.0% NaF ($p<0.05$).

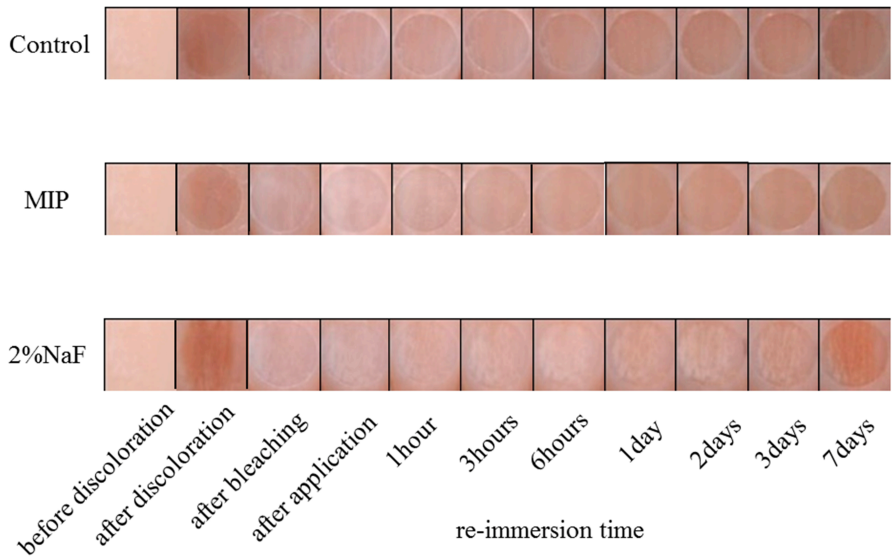

Fig. 2. Typical color change
Photographs showing the typical color change of the specimens before discoloration, after discoloration, after bleaching, after application: 1 hour, 3 hours, and 6 hours; 1 day, 2 days, 3 days, and 7 days

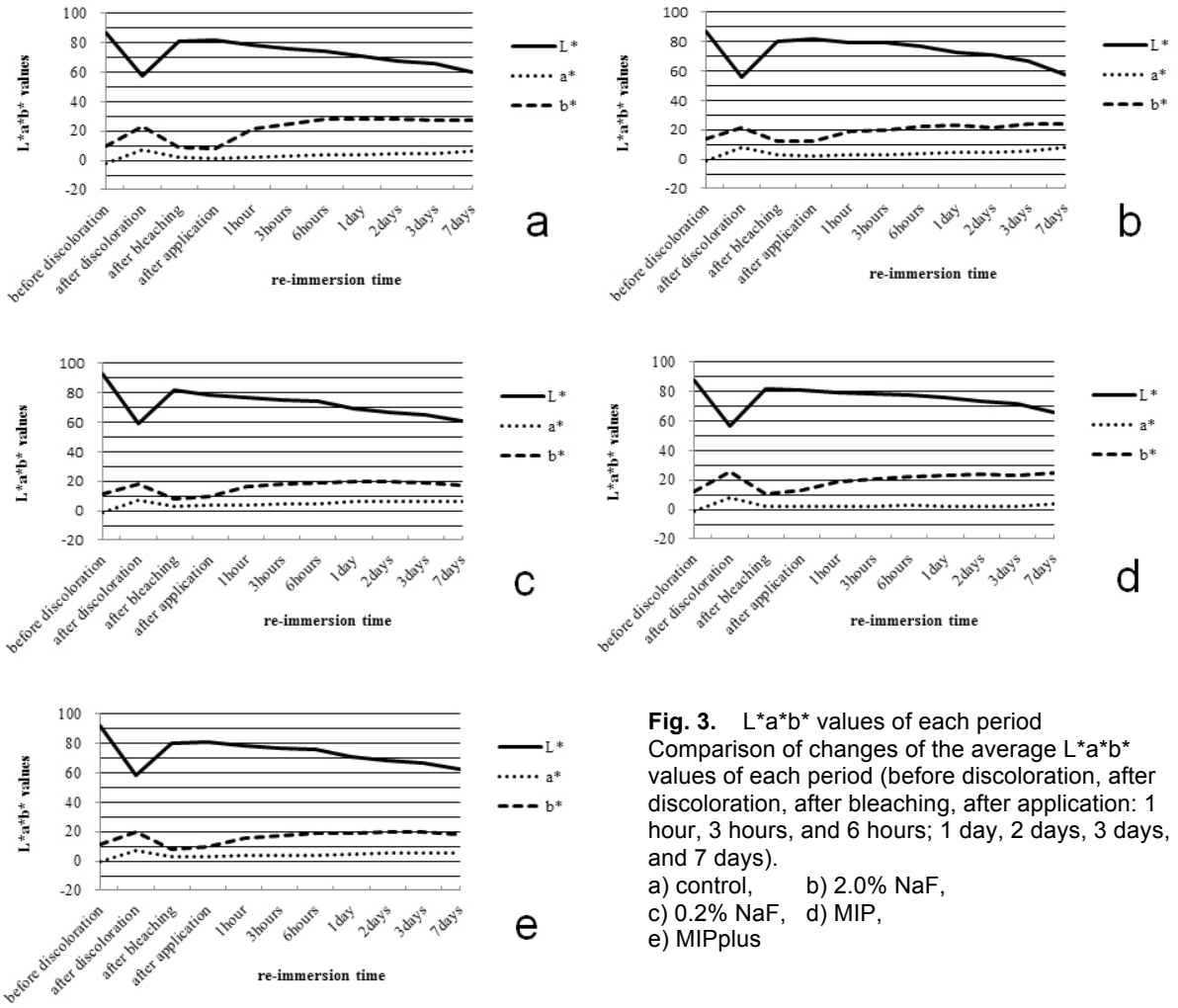

Fig. 3. L*a*b* values of each period
Comparison of changes of the average L*a*b* values of each period (before discoloration, after discoloration, after bleaching, after application: 1 hour, 3 hours, and 6 hours; 1 day, 2 days, 3 days, and 7 days).
a) control, b) 2.0% NaF,
c) 0.2% NaF, d) MIP,
e) MIPplus

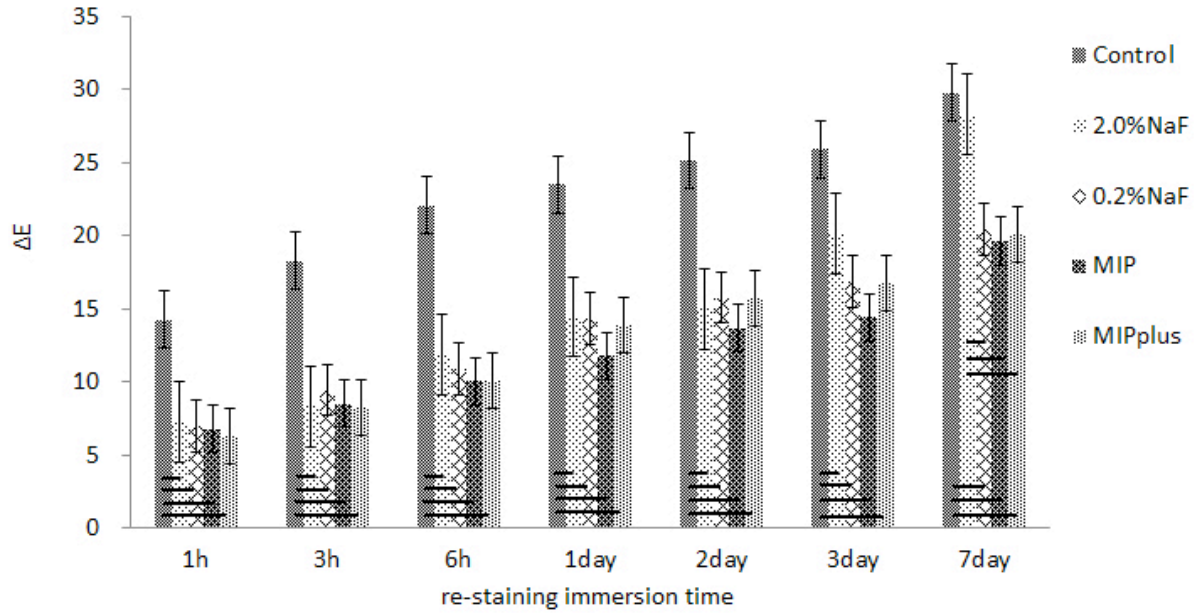

Fig. 4. ΔE change after bleaching
Comparison of changes of ΔE values of each period (before discoloration, after discoloration, after bleaching, after application: 1 hour, 3 hours, and 6 hours; 1 day, 2 days, 3 days, and 7 days).

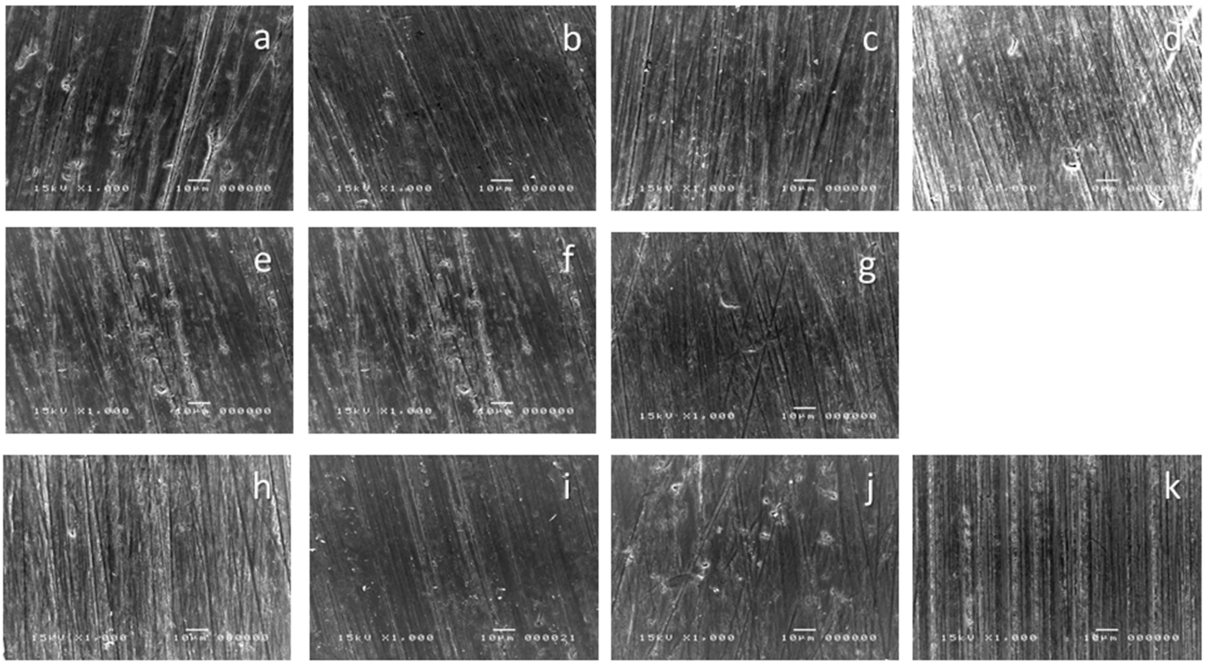

Fig. 5. SEM images of representative surfaces at each stage
A distinct difference could not be observed among groups.
a) typical appearance after initial staining but before bleaching; b) surface after bleaching, and before treatment by experimental agents; c) after application of 2% NaF; d) after application of 0.2% NaF; e) after application of MIP; f) after application of MIPplus; g) control surface after 7-day re-immersion in the staining solution; h) 2% NaF after 7-day re-immersion in the staining solution; i) 0.2% NaF after 7-day re-immersion in the staining solution; j) MIP after 7-day re-immersion in the staining solution; k) MIPplus after 7-day re-immersion in the staining solution.

Under SEM, any morphological changes were not observed before and after bleaching (Fig. 5). Moreover, the application with NaF solutions or CPP-ACP pastes did not appear to affect bleached surfaces (Fig. 5). Although the bleached surfaces were discolored after re-staining for 7 days in all experimental groups, the surface change could not be observed (Fig. 5).

Discussion

The tooth color relapse seems to be one of the biggest problems after tooth bleaching. Therefore, the patient should understand that bleaching is not a permanent treatment and that some periodic re-bleaching will be required.[13] In dental clinics, generally practitioners recommend a re-bleaching treatment about 2 years after the first treatment[5] or once a year;[13] but this time can be even shorter depending on re-staining conditions. The color regression after tooth bleaching is a concomitant phenomenon in the vital teeth bleaching procedure, which is not well-understood by the practitioners yet.[14] The color relapse after bleaching has been investigated in several clinical studies.[5,12,24-27] The degree of the color relapses were varied among them. The color relapse was rarely observed less than one year after bleaching,[12,24] but it was often found later than one year.[5,25]

Many methods have been employed for evaluating bleaching materials and techniques. Several bleaching substrates have been utilized for *in vitro* studies; such as human teeth,[23,28-30] bovine teeth,[29] hematoporphyrin-stained paper[31] and methyleneblue solution.[32] It seems to be very difficult to collect a large number of extracted human incisors as a standard laboratory experiment substrate. In this regard, bovine teeth have been used for many studies concerning bleaching.[21,22,29,33] In the present study, an artificial discolored bovine teeth model which was previously established[22] was employed. The variation in the data obtained could be partially

controlled by initially choosing teeth with a similar degree of discoloration.

There are two known methods for evaluating color change of teeth. One is a subjective method using shade guides and the other is more objective method using a color meter. In this study, the dental color meter was used for measuring tooth color. Tooth discoloration causes can be categorized into intrinsic and extrinsic causes. The intrinsic tooth discoloration can be caused by alkaptonuria, congenital erythropoietic porphyria, congenital hyperbilirubinaemia, amelogenesis imperfecta, dentinogenesis imperfecta, tetracycline staining, fluorosis, enamel hypoplasia, pulpal haemorrhagic products, root resorption, and ageing.[34] The extrinsic discolorations are related to metallic stains as well as non-metallic stains inhaled with air or included in dietary components, beverages, tobacco, mouthrinses and medicaments.[34] In this study, the black tea extract was used for staining the teeth based on the previous works.[23,28,35] This agent is widely available and can cause a reproducible discoloration in a short time.

From the results of this study, re-staining was to some extent prevented in all treatment groups, especially at early periods of immersion. With increased immersion time, the treated surfaces were gradually re-stained. In contrast, in the control group, ΔE value was immediately increased after immersion in the tea extract. Therefore, it can be inferred that re-staining was delayed by the application of CPP-ACP and/or fluoride after tooth bleaching.

It is well known that NaF solution and CPP-ACP paste contribute to remineralization of the decalcified enamel. The CPP-ACP has exhibited anticariogenic potential in laboratory, animal and human *in situ* researches.[19,36,37] In human *in situ* models, the CPP-ACP in sugar-free chewing gum could re-mineralize enamel subsurface lesions,[36] and those re-mineralized enamel surfaces acquired acid resistance.[37] There are also several reports about the effect of CPP-ACP paste on tooth bleaching.[16,38-40] The CPP-ACP paste and CPP-ACP with fluoride paste as well as 250 ppm NaF solution increased the hardness of enamel surface of the bovine incisors bleached with 38% hydrogen peroxide.[38] In line with the results of the present study, it was reported that the treatment of CPP-ACP paste or 1.23% fluoride gel on the enamel of the extracted human incisors after bleaching with 10% carbamide peroxide reduced stain absorption.[16] Moreover, application of CPP-ACP paste on the bovine enamel before and/or after bleaching with 35% hydrogen peroxide or 37% carbamide peroxide prevented negative changes in the roughness and hardness,[40] even after damage exerted by prolonged periods of bleaching.[41] In contrast, it has been reported that the application of CPP-ACP paste did not maintain prevent re-staining and did not recover the damaged enamel surface.[40] The variety of the results seems to be due to different teeth, bleaching methods and experimental protocols. For instance, the acidity and composition of bleaching agent appears to be an important factor affecting bleached enamel surface. It was reported that an acidic bleaching gel significantly reduced bovine enamel microhardness, and the use of remineralizing gels containing fluoride after bleaching could enhance the microhardness of bleached enamel.[42] Another recent study reported that bleaching with an acidic 35% hydrogen peroxide (pH 5.6), even though followed by topical application of fluoride, increased the roughness values and resulted in erosion of enamel of extracted human teeth.[43] Meanwhile, bleaching with a neutral, fluoride-containing hydrogen peroxide gel (38%) did not affect the enamel surface roughness.[44]

The regression of L* value is significantly correlated with the density change of the hard tissue;[14] and the remineralization function of the agent should explain their preventive effects on re-staining of bleached enamel. Nevertheless, the pH of the bleaching material used in this study is balanced around neutral according to the

manufacturer, and no enamel surface erosion or damage could be observed under SEM after bleaching. Therefore, further research should clarify the mechanism of re-staining hindrance observed in this study.

The 2.0% NaF is considered to be a high fluoride concentration and can be applied only in the dental clinic, while 0.2% NaF and CPP-ACP pastes can be used in both dental clinic and home. For office bleaching, fluoride or CPP-ACP paste should be applied immediately after the bleaching procedure. For home bleaching, NaF gel or CPP-ACP paste is recommended to be applied with a bleaching tray after the use of a home breaching gel.

In the current study, application of NaF solution or CPP-ACP paste immediately after tooth bleaching treatment resulted in the prevention of re-staining after tooth bleaching. Further research, especially clinical evaluation is required to clarify the effectiveness of this approach.

Acknowledgements

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References

1. American Dental Association Council on Scientific Affairs. Tooth Whitening/Bleaching: Treatment Considerations for Dentists and Their Patients. American Dental Association 2010; 1-12.
2. Marson FC, Sensi LG, Vieira LC, Araujo E. Clinical evaluation of in-office dental bleaching treatments with and without the use of light-activation sources. Oper Dent 2008; 33: 15-22.
3. Meireles SS, Santos IS, Bona AD, Demarco FF. A double-blind randomized clinical trial of two carbamide peroxide tooth bleaching agents: 2-year follow-up. J Dent 2010; 38: 956-63.
4. Tsubura S. Clinical evaluation of three months' nightguard vital bleaching on tetracycline-stained teeth using Polanight 10% carbamide gel: 2-year follow-up study. Odontology 2010; 98: 134-8.
5. Boushell LW, Ritter AV, Garland GE, Tiwana KK, Smith LR, Broome A, et al. Nightguard vital bleaching: side effects and patient satisfaction 10 to 17 years post-treatment. J Esthet Restor Dent 2012; 24: 211-9.
6. Tredwin CJ, Naik S, Lewis NJ, Scully C. Hydrogen peroxide tooth-whitening (bleaching) products: review of adverse effects and safety issues. Br Dent J 2006; 200: 371-6.
7. Schulte JR, Morrissette DB, Gasior EJ, Czajewski MV. The effects of bleaching application time on the dental pulp. J Am Dent Assoc 1994; 125: 1330-5.
8. Li Y. Safety controversies in tooth bleaching. Dent Clin North Am 2011; 55: 255-63.
9. Tredwin CJ, Naik S, Lewis NJ, Scully C. Hydrogen peroxide tooth-whitening (bleaching) products: review of adverse effects and safety issues. Br Dent J 2006; 200: 371-6.
10. Li Y. Biological properties of peroxide-containing tooth whiteners. Food Chem Toxicol 1996; 34: 887-904.
11. Ziebolz D, Helms K, Hannig C, Attin T. Efficacy and oral side effects of two highly concentrated tray-based bleaching systems. Clin Oral Investig 2007; 11: 267-75.
12. Matis BA, Cochran MA, Eckert G. Review of the effectiveness of various tooth whitening systems. Oper Dent 2009; 34: 230-5.
13. Goldstein RE, Garber DA. Complete Dental Bleaching. 1995; 35-56. Quintessence Publishing, Chicago.
14. Li Q, Xu BT, Li R, Yu H, Wang YN. Quantitative evaluation of colour regression and mineral content change of bleached teeth. J Dent 2010; 38: 253-60.
15. Tschoppe P, Neumann K, Mueller J, Kielbassa AM. Effect of fluoridated bleaching gels on the remineralization of predemineralized bovine enamel in vitro. J Dent 2009; 37: 156-62.
16. Singh RD, Ram SM, Shetty O, Chand P, Yadav R. Efficacy of casein phosphopeptide-amorphous calcium phosphate to prevent stain absorption on freshly bleached enamel: An in vitro study. J Conserv Dent 2010; 13: 76-9.
17. Bizhang M, Seemann R, Duve G, Romhild G, Altenburger JM, Jahn KR, et al. Demineralization effects of 2 bleaching procedures on enamel surfaces with and without post-treatment fluoride application. Oper Dent 2006; 31: 705-9.
18. Attin T, Kielbassa AM, Schwanenberg M, Hellwig E. Effect of fluoride treatment on remineralization of bleached enamel. J Oral Rehabil 1997; 24: 282-6.
19. Reynolds EC. Calcium phosphate-based remineralization systems: scientific evidence?. Aust Dent J 2008; 53: 268-73.
20. Hamba H, Nikaido T, Inoue G, Sadr A, Tagami J. Effects of CPP-ACP with sodium fluoride on inhibition of bovine enamel demineralization: a quantitative assessment using micro-computed tomography. J Dent 2011; 39: 405-13.
21. Kwon YH, Huo MS, Kim KH, Kim SK, Kim YJ. Effects of hydrogen peroxide on the light reflectance and morphology of bovine enamel. J Oral Rehabil 2002; 29: 473-7.
22. Kishi A, Otsuki M, Sadr A, Ikeda M, Tagami J. Effect of light units on tooth bleaching with visible-light activating titanium dioxide photocatalyst. Dent Mater J 2011; 30: 723-9.
23. Sulieman M, Addy M, Rees JS. Development and evaluation of a method in vitro to study the effectiveness of tooth bleaching. J Dent 2003; 31: 415-22.
24. Meireles SS, dos Santos Ida S, Della Bona A, Demarco FF. A double-blind randomized controlled clinical trial of 10 percent versus 16 percent carbamide peroxide tooth-bleaching agents: one-year follow-up. J Am Dent Assoc 2009; 140: 1109-17.
25. Grobler SR, Hayward R, Wiese S, Moola MH, van W Kotze TJ. Spectrophotometric assessment of the effectiveness of Opalescence PF 10%: a 14-month clinical study. J Dent 2010; 38: 113-7.
26. Turkun M, Celik EU, Aladag A, Gokay N. One-year clinical evaluation of the efficacy of a new daytime at-home bleaching technique. J Esthet Restor Dent 2010; 22: 139-46.

27. Giachetti L, Bertini F, Bambi C, Nieri M, Scaminaci Russo D. A randomized clinical trial comparing at-home and in-office tooth whitening techniques: A nine-month follow-up. J Am Dent Assoc 2010; 141: 1357-64.
28. Sulieman M, Addy M, MacDonald E, Rees JS. The effect of hydrogen peroxide concentration on the outcome of tooth whitening: an in vitro study. J Dent 2004; 32: 295-9.
29. Joiner A. The bleaching of teeth: a review of the literature. J Dent 2006; 34: 412-9.
30. Lima DA, Aguiar FH, Liporoni PC, Munin E, Ambrosano GM, Lovadino JR. In vitro evaluation of the effectiveness of bleaching agents activated by different light sources. Int J Prosthodont 2009; 18: 249-54.
31. Suemori T, Kato J, Nakazawa T, Akashi G, Igarashi A, Hirai Y, Kumagai Y, et al. Effects of light irradiation on bleaching by a 3.5% hydrogen peroxide solution containing titanium dioxide. Laser Phys 2008; 5: 379-83.
32. Suyama Y, Otsuki M, Ogisu S, Kishikawa R, Tagami J, Ikeda M, et al. Effects of light sources and visible light-activated titanium dioxide photocatalyst on bleaching. Dent Mater J 2009; 28: 693-9.
33. Wetter NU, Barroso MC, Pelino JE. Dental bleaching efficacy with diode laser and LED irradiation: an in vitro study. Laser Surg Med 2004; 35: 254-8.
34. Watts A, Addy M. Tooth discolouration and staining: a review of the literature. Br Dent J 2001; 190: 309-16.
35. Sulieman M, Addy M, Macdonald E, Rees JS. The bleaching depth of a 35% hydrogen peroxide based in-office product: a study in vitro. J Dent 2005; 33: 33-40.
36. Shen P, Cai F, Nowicki A, Vincent J, Reynolds EC. Remineralization of enamel subsurface lesions by sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. J Dent Res 2001; 80: 2066-70.
37. Iijima Y, Cai F, Shen P, Walker G, Reynolds C, Reynolds EC. Acid resistance of enamel subsurface lesions remineralized by a sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. Caries Res 2004; 38: 551-6.
38. Bayrak S, Tunc ES, Sonmez IS, Egilmez T, Ozmen B. Effects of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) application on enamel microhardness after bleaching. Am J Dent 2009; 22: 393-6.
39. Kim YS, Kwon HK, Kim BI. Effect of nano-carbonate apatite to prevent re-stain after dental bleaching in vitro. J Dent 2011; 39: 636-42.
40. Cunha AG, De Vasconcelos AA, Borges BC, Vitoriano Jde O, Alves-Junior C, Machado CT, et al. Efficacy of in-office bleaching techniques combined with the application of a casein phosphopeptide-amorphous calcium phosphate paste at different moments and its influence on enamel surface properties. Microsc Res Tech 2012; 75: 1019-25.
41. Alkhtib A, Manton DJ, Burrow MF, Saber-Samandari S, Palamara JE, Gross KA, et al. Effects of bleaching agents and Tooth Mousse on human enamel hardness. J Investig Clin Dent 2013; 4: 94-100.
42. Borges AB, Yui KC, D'Avila TC, Takahashi CL, Torres CR, Borges AL. Influence of remineralizing gels on bleached enamel microhardness in different time intervals. Oper Dent 2010; 35: 180-6.
43. Dominguez JA, Bittencourt B, Michel M, Sabino N, Gomes JC, Gomes OM. Ultrastructural evaluation of enamel after dental bleaching associated with fluoride. Microsc Res Tech 2012; 75: 1093-8.
44. Pedreira De Freitas AC, Botta SB, Teixeira Fde S, Salvadori MC, Garone-Netto N. Effects of fluoride or nanohydroxiapatite on roughness and gloss of bleached teeth. Microsc Res Tech 2011; 74: 1069-75.

Correspondence to:

Dr. Masayuki Otsuki
Cariology and Operative Dentistry, Department of Oral Health Sciences,
Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University.
1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Japan.
Fax: +81-3-5803-0195 E-mail: otsuki.ope@tmd.ac.jp

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