Dental Materials Journal 2013; 32(5): 761–766

Efficacy of cold light bleaching using different bleaching times and their effects on human enamel

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This study investigated the efficacy of cold light bleaching using different bleaching times and the effects thereof on tooth enamel. Before and after bleaching, stained tooth specimens were subjected to visual and instrumental colorimetric assessments using Vita Shade Guide and spectrophotometric shade matching. Enamel surface alterations were examined using scanning electron microscopy (SEM) to analyze surface morphology, surface microhardness (SMH) measurement to determine changes in mechanical properties, and X-ray diffraction (XRD) to characterize *post*-bleaching enamel composition. Cold light bleaching successfully improved tooth color, with optimal efficacy when bleaching time was beyond 10 min. Significant differences in surface morphology were observed among the different bleaching times, but no significant differences were observed for enamel composition and surface microhardness among the different bleaching times. Results of this study revealed an association between the bleaching time of cold light bleaching and its whitening efficacy. Together with the results on enamel surface changes, this study provided positive evidence to support cold light bleaching as an in-office bleaching treatment.

Keywords: Cold light bleaching, Bleaching time, Shade matching, Enamel

INTRODUCTION

The causes of tooth discoloration are usually various and multifaceted, and they have been classified as extrinsic, intrinsic, and internalized discoloration[1]. In tandem with technological advances in dentistry, there is also an increase in patient awareness of the ability to improve the appearance of their discolored teeth. Not only are these patients seeking to improve the esthetic appearance of their smiles, they are also seeking an effective method. There are several ways to manage tooth discoloration, which include crowns, veneers, or tooth bleaching. For crowns and veneers, these treatment options entail a moderate loss of dental hard tissue. Vital tooth bleaching is not only a less costly alternative to bonded restorative dentistry, it is a conservative and non-invasive technique which has been well accepted to be safe and effective[2].

Tooth bleaching using oxalic acid was first introduced in 1848[3], followed by hydrogen peroxide (HP) in 1884[4]. Contemporary tooth bleaching systems are primarily based on oxidation by HP or one of its precursors, carbamide peroxide (CP)[5], and they can be applied at home (home bleaching) or in a dental office by a professional dentist (in-office bleaching)[6]. In-office bleaching has become a widely used procedure because of these advantages: minimally invasive, immediate visible results, and no need of patient cooperation[7]. A novel in-office bleaching method is cold light bleaching which uses blue LED light.

Tooth bleaching efficacy is influenced by several factors: type of bleaching system, concentration of the bleach, duration of bleaching time, light or heat application. In the case of cold light bleaching, light is used to activate peroxide to accelerate the chemical redox reactions of the bleaching process[8]. While bleaching time is one key factor in determining the overall tooth whitening efficacy from peroxide-containing products, the effects of different bleaching times of cold light bleaching on tooth color and enamel properties are still unknown[9].

To evaluate the efficacy of different tooth bleaching methods, researchers have developed several *in vitro* tooth staining models by immersing extracted teeth in hemolysate, tea, chlorhexidine, or human saliva to stain the teeth[10-13]. After bleaching, different methods have been employed to evaluate the effects of bleaching on teeth. Scanning electron microscopy (SEM) is a rapid and convenient method for qualitative analysis of the surface morphology of enamel specimens, while surface microhardness (SMH) measurement is a simple method to determine the mechanical properties of enamel specimens[14]. X-ray diffraction (XRD) or SEM is typically used to characterize the chemical composition of enamel after bleaching[15]. To date, the effects of peroxide-based products on dental ultrastructure remain controversial. While some authors reported that no adverse effects were observed, others claimed reduction in calcium-phosphate ratio and loss of organic components from treated enamel surfaces. Nonetheless, it is highly probable that low pH and hydrogen peroxide oxidation could lead to ultrastructural changes in dentin during internal dental bleaching[16,17].

The purpose of the current study was to compare the efficacies of cold light bleaching of different bleaching durations and the effects thereof on tooth enamel, and thereby identify the optimal bleaching time for cold light bleaching. To evaluate the efficacy of cold

Received Apr 15, 2013: Accepted Jul 4, 2013
doi:10.4012/dmj.2013-109 JOI JST.JSTAGE/dmj/2013-109

Table 1 Standard Vita Shade Guide with 16 shades ranked from the lightest color on the left to the darkest color on the right

B1	A1	B2	D2	A2	C1	C2	D4	A3	D3	B3	A3.5	B4	C3	A4	C4
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

light bleaching, color changes of enamel were assessed visually and instrumentally using Vita Shade Guide and a spectrophotometer. To investigate the effects of different bleaching times on enamel surface alterations, SEM and surface microhardness measurement were used to analyze the surface morphology and assess the mechanical properties of treated enamel surfaces.

MATERIALS AND METHODS

Specimen preparation

Fifty premolars, freshly extracted for orthodontic reasons and free from enamel surface abnormalities such as cracks or fractures, caries and other defects, were immersed in 0.9% sodium chloride (NaCl). The teeth were acquired after obtaining informed consent from patients. After the selected teeth were cleaned using curettes, they were sectioned using a water-cooled diamond wafering blade at the cement-to-enamel junction, discarding the root portion. The crown portion of each tooth was sectioned in half mesiodistally for this study.

A tea solution was prepared by boiling 2 g of tea (Extra Strong Black Tea, Lipton, Anhui, China) in 100 mL of distilled water. After 5 min of infusion, the tea was filtered and the solution was recovered. All specimens were immersed in this standard tea solution for 24 h at room temperature in screw-capped, plastic, universal containers. After staining, each specimen was embedded in a self-curing resin block with the sectioned surface facing upwards.

Stained and resin-embedded specimens were randomly allocated into five groups (n=10). Control group (group A) specimens were immersed in 100 mL of distilled water for 1 h. Specimens in groups B, C, D, and E were subjected to cold light bleaching (Beyond II, Beyond Technology, Centennial, CO, USA) using a standard cold light bleaching procedure for 8, 10, 15, and 20 min respectively. This procedure was performed three times.

Color evaluation

Before and after bleaching, the shade of each stained specimen was assessed using two different shade assessment methods: standard Vita Shade Guide *versus* a spectrophotometer.

1. Standard Vita Shade Guide

This is a visual and subjective assessment method. One investigator conducted all the shade comparisons using a standard Vita Shade Guide (Vita Zahnfabrik, Germany) before and after bleaching. During assessment, all the specimens were laid on a black background. Shade guide tabs were arranged from B1 to C4, each corresponding to a numerical value from 1 to 16 (Table 1).

2. Spectrophotometer

This is an instrumental method for shade matching. The shade matching device used in this study was Olympus Crystaleye (Olympus, Tokyo, Japan). This dental spectrophotometer combined the benefits of a traditional spectrophotometer with digital photography. Using a 7-band LED light source, the images produced by Crystaleye could depict tooth colors more precisely than conventional systems used with digital cameras. Color measurements were performed with specimens held in homemade embedding cassettes.

Spectrophotometric color measurement of specimens was based on the CIE $L^*a^*b^*$ system. The $L^*a^*b^*$ system organizes all existing colors within a three-dimensional color space. L^* represents the degree of lightness and ranges from 0 (black) to 100 (white); a^* represents the green-red axis while b^* represents the blue-yellow axis. Color measurement was performed at the middle region of each specimen. Color changes after bleaching were expressed as ΔL^*, Δa^*, Δb^*. Overall color difference of the specimens in each group (ΔE^*) was calculated using the following formula[16]:

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

Color measurement results obtained from standard Vita Shade Guide and Olympus Crystaleye spectrophotometer were analyzed using SPSS 16.0 for Windows. Data were presented as mean±SD, and one-way analysis of variance (ANOVA) was used to determine the variation among the means of five test groups at p=0.05.

Scanning electron microscopy (SEM)

In each group, dental slabs (3×3×3 mm) were obtained from the middle third of each tooth using a low-speed diamond saw under water cooling. After rinsing and drying, enamel surface morphology of each specimen was observed using an SEM (Superscan SSX-550, Shimadzu, Japan).

X-ray diffraction (XRD) analysis

In each group, enamel layer was obtained from the middle of each tooth using a low-speed diamond saw under water cooling. After rinsing and drying, enamel specimens were ground to produce a fine powder and then examined using an XRD (X'Pert Pro MPD, PW 3040/60, The Netherlands).

Surface microhardness (SMH) measurement
An additional 15 dental slabs (3×3×3 mm) were prepared from freshly extracted premolars using a low-speed diamond saw under water cooling. The labial surfaces of specimens were polished, and the specimens were randomly divided into five groups (n=3).

Surface microhardness measurements were performed before and after bleaching using a microhardness tester (Q10A, Qness, Austria) under a load of 100 g for 10 s. Five Vickers indentations were made on each specimen. For statistical analysis of microhardness values, the mean values of five measurements of each group were statistically analyzed by one-way ANOVA at p=0.05.

RESULTS

Color change
Table 2 shows the mean color change results of 50 specimens measured according to the Vita Shade Guide. Color change became more visibly obvious as bleaching time increased from Group A to Group E. Significant differences were found when comparing Groups B, C, D, and E to Group A (p<0.05). Statistically significant differences were also observed when comparing long-duration groups (*i.e.*, Groups C, D, and E with bleaching time of more than 8 min) to Group B (8-min bleaching time) (p<0.05). Interestingly, no statistically significant differences were observed among the long-duration groups, *i.e.*, Groups C, D, and E with bleaching time of more than 8 min.

Figure 1 shows the color change results measured using the spectrophotometer. For control group A, color change was hardly perceptible as indicated by the negligible results of ΔL^*, Δa^*, Δb^*, and ΔE^*. However, comparison of ΔL^*, Δa^*, Δb^*, and ΔE^* revealed significant differences between control group A and the bleached groups (p<0.05). Among the bleached groups, significant differences were observed between long-durations groups (Groups C, D, and E) to Group B (p<0.05), but no significant differences were found among the long-duration groups (Groups C, D, and E) (p>0.05).

Color change results obtained using the spectrophotometer were consistent with those of Vita Shade Guide. Therefore, cold light bleaching caused perceptible tooth color change and that bleaching time beyond 10 min heightened its efficacy.

Enamel surface morphology
Figure 2 shows the SEM images of the enamel surfaces of Groups A to E. When compared to control group A (Fig. 2a), bleached groups showed apparent changes in enamel surface roughness. Varying degrees of surface changes in terms of porosities, depressions, and superficial irregularities were observed for the bleached groups (Figs. 2b, c, d, e).

Enamel composition
Figure 3 shows the XRD patterns of different bleaching times with indexed peak positions. In Group A, typical hydroxyapatite and fluorapatite peaks were observed. After bleaching, Groups B, C, D, and E showed similar peaks as Group A.

Enamel surface microhardness
Table 3 presents the mean Vickers hardness values of each group before and after bleaching. Although microhardness decreased with bleaching treatment, there were no significant differences in microhardness among all the five groups (p>0.05).

Table 2 Color change results of different bleaching times as measured by Vita Shade Guide

Group	Mean	S.D.	Range	Statistical analysis
A	0.00	0.00	0.00	
B	5.00	0.82	4–6	a^*
C	7.4	1.51	6–10	a^*, b^*
D	7.6	1.51	6–10	a^*, b^*
E	7.9	1.43	6–10	a^*, b^*

A: Immersion in distilled water only for 1 h. B, C, D, E: Bleaching time of 8 min, 10 min, 15 min and 20 min respectively per treatment, and treatment was performed three times.
a^*: Significant difference between Groups B, C, D, E to Group A (p<0.05).
b^*: Significant difference between Groups C, D, E to Group B (p<0.05), but no significant difference among Group C, D, and E.

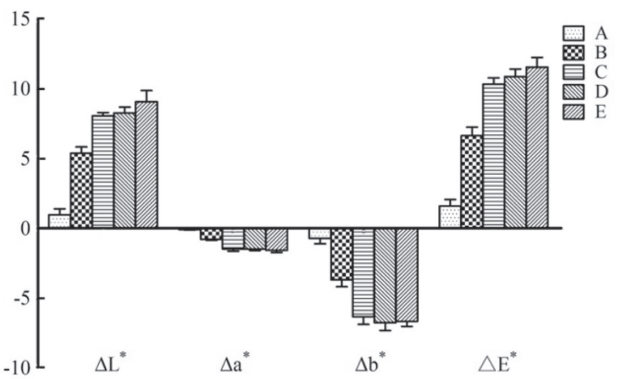

Fig. 1 Color change results of different bleaching times as measured using a spectrophotometer and expressed in ΔL^*, Δa^*, Δb^*, and ΔE^*.
A: Immersion in distilled water only for 1 h.
B, C, D, E: Bleaching time of 8 min, 10 min, 15 min and 20 min respectively per treatment, and treatment was performed three times.

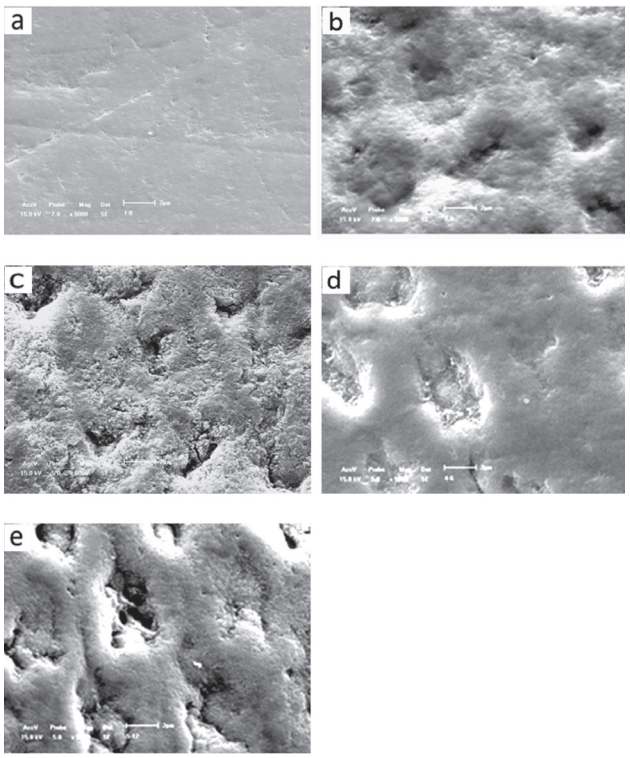

Fig. 2 SEM images of enamel surfaces according to different bleaching times.
A: Immersion in distilled water only for 1 h.
B, C, D, E: Bleaching time of 8 min, 10 min, 15 min and 20 min respectively per treatment, and treatment was performed three times.

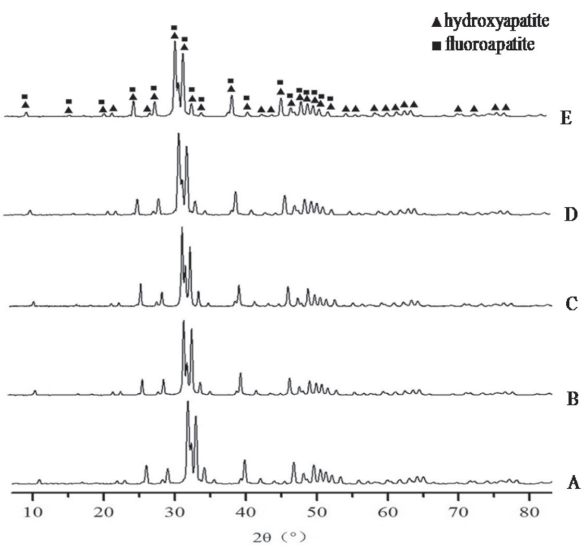

Fig. 3 XRD patterns according to different bleaching times.
A: Immersion in distilled water only for 1 h.
B, C, D, E: Bleaching time of 8 min, 10 min, 15 min and 20 min respectively per treatment, and treatment was performed three times.

Table 3 Mean Vickers hardness number (±SD) before and after cold light bleaching

Group	Before bleaching	After bleaching
A	384.86±0.84	384.14±0.60
B	386.43±2.32	384.72±1.21
C	387.08±1.89	385.16±0.30
D	384.48±4.80	382.08±5.56
E	386.67±1.58	384.09±1.19

A: Immersion in distilled water only for 1 h. B, C, D, E: Bleaching time of 8 min, 10 min, 15 min and 20 min respectively per treatment, and treatment was performed three times

DISCUSSION

In this study, the tooth staining model was based on immersion of extracted teeth in tea. This model has been used previously and favorably reported for its two key advantageous features: good reproducibility of stained teeth for tooth color measurements whereby tooth discoloration changes can be objectively measured using a spectrophotometer[17-19]. After immersion in tea for 24 h, the stained teeth of this study were verified to have similar tooth color change results.

Many contemporary in-office tooth bleaching systems are activated by light, such as blue LED light used in cold light bleaching[16]. Although clinical data has shown that cold light bleaching is effective in removing extrinsic and intrinsic stains, more clinical research is needed to investigate the relationship between bleaching time and whitening efficacy and the effects of different bleaching times on dental hard tissues. Results of the present study showed that there were statistically significant differences in whitening efficacy among different bleaching times. Moreover, this study provided the needed data for the optimal bleaching time.

One-way ANOVA was used to statistically analyze the colorimetric measurement results according to Vita Shade Guide and spectrophotometric shade matching (ΔL^*, Δa^*, Δb^*, and ΔE^* values). There were significant differences in color change results not only between the non-bleached control group and the bleached groups, but whitening efficacy was significantly heightened when bleaching time was beyond 10 min. Cold light technology activates bleaching agents diffused into enamel and dentin by causing hydrogen peroxide (HP) to oxidize a variety of organic and inorganic colored compounds, leading to reduction in tooth color[13]. Therefore, a longer bleaching time would allow bleaching agents to react more thoroughly with colored compounds. However, in-office bleaching time is limited in the clinical setting. Results of the present study recommended an optimal bleaching time of beyond 10 min.

SEM micrographs of enamel specimens showed that 1-h immersion in distilled water did not cause any changes to surface morphology. After bleaching treatment with 35% hydrogen peroxide, SEM observation revealed that a longer bleaching time resulted in more morphological alterations in superficial enamel. Our results agreed with those of Pinto *et al.*, who reported that exposure to 35% hydrogen peroxide increased enamel surface roughness and significantly altered the superficial morphology of enamel[20]. Morphological changes observed in this study included surface erosion, depressions, and porosities. In a study by Shi *et al.*, demineralization in the enamel surface after cold light bleaching was attributed to the acidic erosive action of peroxide-containing bleaching agent[21]. Interestingly, contradicting results were obtained with carbamide peroxide (CP). On the one hand, it was reported that high-concentration carbamide peroxide increased enamel surface roughness[22]. On the other hand, it was reported that enamel surface showed no morphological changes following bleaching *in situ* with carbamide peroxide agents[23].

Hydroxyapatite peaks in the XRD patterns of the bleached groups were similar to those of the control group. Another XRD study also showed that hydroxyapatite was not affected by 30% H_2O_2 treatment[24]. With carbamide peroxide, energy dispersive X-ray (EDX) analysis showed that home bleaching with 15% to 16% carbamide peroxide did not harm enamel chemical composition[23].

Peroxide-containing bleaching agents of different concentrations (10% CP, 7.5% HP, 37% CP, 35% CP, and 35% HP) were found to cause a reduction in enamel microhardness[20], even with home bleaching agents of lower CP or HP concentrations[25]. However, bleaching with a simulated body fluid (SBF)-containing bleaching agent caused tooth surface microhardness to increase as compared to treatment with distilled water[26]. This was probably due to the deposition of bone-like apatite[26]. It was also shown bleaching with HP- or CP-containing bleaching agents decreased dentin microhardness[27,28]. In a study by Chng *et al.*, 7-day intracoronal bleaching with 30% hydrogen peroxide sealed in the root canal significantly weakened the outer layer of dentin[29]. In the present study, there were no significant differences in enamel microhardness between the control group and bleached groups after treatment. There were also no significant differences among the bleached groups in terms of the effect of bleaching time on enamel microhardness.

CONCLUSIONS

Within the limitations of the current study, the following conclusions were drawn:

1. Cold light bleaching successfully improved tooth color and that whitening efficacy was heightened when bleaching time was beyond 10 min.
2. Alterations in enamel surface morphology were observed after cold light bleaching treatment, but enamel composition and surface microhardness were not affected.

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