Effect of ultraviolet ray on tooth bleaching using titanium dioxide photocatalyst

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Abstract
Purpose: The purpose of this study was to evaluate the effect of ultraviolet ray (UV) on tooth bleaching using titanium dioxide photo catalyst in vitro.
Materials and Methods: Hematoporphyrin-stained paper (HSP) and artificially discolored bovine teeth (BT) were bleached by an in-office bleaching material containing low concentration of hydrogen peroxide and titanium dioxide photocatalyst. For the bleaching, light irradiation was performed at wavelengths of 265, 310, 365, 405, and 450 nm for HSP, and 265, 300, and 405+470 nm for BT. Before and after bleaching, color was measured to obtain CIE L*a*b* values. The color differences (ΔE) were calculated and were statistically analyzed.
Results: In the HSP experiment, the 265 and 310 nm groups showed a remarkable bleaching effect and ΔE of the 310 nm group was statistically the highest (p < 0.05). In the BT experiment, ΔE of the 265 nm group was statistically the highest followed by 300 nm, then 405+470 nm groups (p < 0.05).
Conclusion: UV-C (265 nm) and UV-B (300 and 310 nm) showed high bleaching effect with an in-office bleaching material containing low concentration of hydrogen peroxide and titanium dioxide photocatalyst.

Key Words: color measurement, hydrogen peroxide, photocatalyst, tooth bleaching, ultraviolet ray

Introduction
Tooth bleaching is one of the least invasive and most conservative esthetic dental treatments and can enhance a person’s smile. Tooth bleaching for vital teeth is categorized into two methods types: as at-home bleaching and in-office bleaching. In both methods, the bleaching materials contain peroxides as an active ingredient. At-home bleaching is applied by the patient at home with a custom tray and bleaching gel which were prescribed by a dentist. Generally, at-home bleaching material contains 10% or 14% carbamide peroxide and in-office bleaching products contain high concentration of hydrogen peroxide. In-office bleaching is applied by a dentist in a dental office.

The ideal is to bleach the tooth safely in a short time. There are many factors which affect in-office bleaching; such as the concentration of hydrogen oxide [1], pH of the bleaching material [2], the catalyst [3,4], bleaching duration and cycles [5], and temperature [6]. Bleaching materials containing higher concentration of hydrogen peroxide show higher bleaching effect and higher toxicity. Lower concentrations are safer. However, bleaching materials containing low concentrations of hydrogen peroxide do not normally bleach teeth adequately. Light irradiation can be expected to increase the temperature of the bleaching material and to accelerate the reaction through photocatalyst. The bleaching effect with the reaction of photocatalyst was varied depending on the wavelength and intensity of the light [7,8]. Almost all of light units for tooth bleaching have used various wavelengths of visible light.

One bleaching material contains low concentration of hydrogen peroxide and titanium dioxide photocatalyst. This photocatalyst was effective with short wavelength of visible light. However, there has been no study on evaluation of this bleaching material with ultraviolet ray.

The purpose of this study was to evaluate the effect of ultraviolet ray on tooth bleaching using a titanium dioxide photo catalyst in vitro.

Materials and Methods
Tooth bleaching material and ultraviolet lights
An in-office bleaching material (Pyrenees, Nissin Dental Products, Kyoto, Japan) was used for this study. This bleaching material consists of two solutions. One solution contains hydrogen peroxide, stabilizer, pH conditioner and purified water, whereas the other one contains titanium dioxide, thickener and purified water. The titanium dioxide in the solution works as a photocatalyst. After mixing, the concentration of hydrogen peroxide was 3.5% and pH was 6.0.

The characteristics of experimental light emitting diodes (LEDs) used in this study were shown in Table 1. For the experiment using hematoporphyrin-stained paper (HSP), LED lights of wavelengths of 265, 310, 365, 405, and 450 nm...
were used. For the stained bovine teeth (BT) experiment, LED lights of wavelength of 265, 300, and 405+470 nm were used. Different lights were used for the HSP and BT experiments because of a technical reason. The illuminated area of LEDs used for the HSP is too small for the BT experiment. For the BT experiment, LEDs of higher intensity had to be used. The intensity of the light was adjusted by changing the electric current and the distance between the light and the target.

| Wavelength (nm) | Intensity (mW/cm²) | Irradiation time (min) |
|-----------------|--------------------|-----------------------|
| 265 UVC         | 10.5               | 20                    |
| 310 UVB         | 10.1               | 20                    |
| HSP 365 UVA     | 10.8               | 20                    |
| 405 visible light (violet) | 10.3 | 20 | |
| 450 visible light (blue) | 10.9 | 20 | |
| 265 UVC         | 3.12               | 10 (per 1 cycle)      |
| BT 300 UVB      | 3.17               | 10 (per 1 cycle)      |
| 405+470 visible light (blue)* | 3 | 10 (per 1 cycle) |

HSP, experiment using hematoporphyrin-stained paper; BT, experiment using stained bovine teeth
*LED unit (G-Light Prima II plus) at “20” mode

Experiment using hematoporphyrin-stained paper (HSP)
The 0.24 g of hematoporphyrin (Wako Pure Chemical, Tokyo, Japan) was dissolved in 300 mL of ethanol to prepare 0.1 wt% hematoporphyrin ethanol solution. Photo-printing papers for an ink-jet printer were immersed in the solution for 10 min. Then, the stained paper was dried in a dark room at room temperature and was cut into small pieces with approximately 2 × 2 cm. The color of each HSP was measured by a colorimeter (NR-11, Nippon Denshoku, Tokyo, Japan) and CIE L*a*b* values were obtained. The samples which L* values shown 25-29 were selected and used for the experiment.

The two solutions of Pyrenees were well mixed. The 5 μL of the mixed solution was applied on the HSP and each light was exposed for 20 min. Ten specimens were bleached in each group (n = 10). After light irradiation, the bleaching material was removed. Before and after the bleaching procedure, a photograph was taken with a digital camera and the color of the HSP surface was measured by a CIE XYZ camera (RC500, PaPaLaB, Hamamatsu, Japan) and CIE L*a*b* values were obtained. The color difference (ΔE) was calculated using the following equation.

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

\[
\Delta L^* = (L^* \text{ value after bleaching}) - (L^* \text{ value before bleaching})
\]

\[
\Delta a^* = (a^* \text{ value after bleaching}) - (a^* \text{ value before bleaching})
\]

\[
\Delta b^* = (b^* \text{ value after bleaching}) - (b^* \text{ value before bleaching})
\]

Experiment using stained bovine teeth (BT)
Frozen extracted bovine incisors were purchased from a slaughterhouse. They were thawed with tap water and soft tissue remnant was removed by a scalpel. The labial surface was ground by #600 and #800 grit silicon carbide papers to obtain a flat surface with 1 mm enamel thickness. The specimen with approximately 6 × 6 mm was obtained by cutting with a slow speed diamond saw. Each specimen was then embedded in a cylindrical plastic tube (10 mm internal diameter and 10 mm height) with a dental self-polymerizing resin (UniFast III clear, GC, Tokyo, Japan). The exposed enamel surface was further polished with #1,000 and #1,200 grit silicon carbide papers.

Tea extract was obtained by means of immersion of two tea bags of black tea (Lipton Yellow Label, Unilever Japan, Tokyo, Japan) in 200 mL of hot water for 10 min and the extract was used as dye solution. The bovine teeth specimens were immersed in the dye solution for 7 days at 37°C and solution was changed at fourth day. After staining, the color of the enamel surfaces of the specimens was measured by a colorimeter and the specimens with L* values 22-45 were employed for this study. The mixed bleaching solution (Pyrenees) was applied on the enamel surface of the specimen and ultraviolet LED light with wavelength of 265 or 300 nm was irradiated for 10 min. Ten samples were bleached for each ultraviolet LED light (n = 10). Bleaching procedure was repeated 6 times. Before bleaching and after each bleaching procedure, photo was taken and color was measured by a colorimeter (NR-11). Color differences between before
bleaching and after each bleaching step were calculated as same as HSP experiment. Additionally, a visible light LED light unit (G-light Prima II plus, GC) was used as a control. This light unit has one violet LED (405 nm) and three blue LEDs (470 nm). Light intensity of the light unit was adjusted by means of the distance between the light chip and irradiated enamel surface.

Statistical analysis
The equality of variances of ΔL*, Δa*, Δb*, and ΔE values in each group was tested by Levene's test. For HSP experiment obtained data was analyzed by one-way analysis of variance (ANOVA) at confidence level of 0.05% (α = 0.05). For BT experiment, the data was analyzed by two-way ANOVA with factors of “wavelength” and “bleaching times” followed by one-way ANOVA. For both HSP and BT groups, if the normality was equal, data was further analyzed by Tukey's honestly significant difference (HSD) test. If normality was not equal, Dunnett T3 test was used. A commercially available software (IBM SPSS Statistics ver. 24, IBM, Armonk, NY, USA) was used for those analyses.

Results
Typical images after bleaching of HSP experiment are shown in Fig. 1. The 265 and 310 nm groups showed remarkable bleaching effect than the other groups. The bleached surface of 265 nm group was not uniform. The ΔL*, Δa*, Δb*, and ΔE of HSP experiment are presented in Table 2. Generally, L* value was increased, and a* and b* values were decreased. The 265 and 310 nm groups showed high ΔL* values and 265 nm group showed the statistically highest ΔE (p < 0.05).

![Typical images of HSP specimens after bleaching](image_url)

Table 2 Materials used ΔL*, Δa*, Δb*, and ΔE of HSP experiment

| Material | ΔL*     | Δa*      | Δb*      | ΔE       |
|----------|---------|----------|----------|----------|
| 265      | 3.05 (0.80) ab | -3.14 (0.66)c | 0.34 (0.89)g | 4.53 (0.75)i |
| 310      | 3.56 (1.36)a  | -5.03 (0.69)d | 0.01 (1.03)f | 6.29 (1.29)b  |
| 365      | 2.33 (1.22)abc | -3.45 (1.00)c | -0.16 (1.18)g | 5.43 (0.83)j  |
| 405      | 2.15 (0.75)bc | -3.29 (1.15)c | -0.05 (1.03)g | 5.03 (1.04)j  |
| 450      | 1.56 (0.69)c  | -3.25 (1.10)c | -0.46 (1.01)g | 4.77 (0.69)j  |

Same superscripts in a column mean no statistical difference (p > 0.05)

Typical image at each bleaching period in each group of BT experiment is shown in Fig. 2. The 265 and 300 nm groups showed higher bleaching effect than 405+470 nm group gradually. The changes of ΔL*, Δa*, Δb*, and ΔE of BT experiment are shown in Fig. 3. ΔL* of 265 nm group was statistically highest followed by 300, then 405+470 groups (p < 0.05).
Hematoporphyrin-stained paper (HSP) and artificial discolored bovine tooth (BT) were used in this study. Both methods were widely used for evaluating bleaching effects in vitro. The trauma sometimes causes the discolored teeth. Those teeth were stained with blood break down products [9]. Hematoporphyrin is a blood pigment and hematoporphyrin and its derivatives are among the chromogens of discolored teeth. Hematoporphyrin-stained paper (HSP) is easy to prepare and tests using HSP are sensitive and highly reproducible. This evaluation method is thought to be suitable as a screening test for bleaching effectiveness. Although evaluation using HSP is simple and easy, the results are not straightforwardly connected to clinical results. Before clinical application, it is necessary to evaluate the bleaching effect in extracted teeth. Extracted human teeth are most suitable and reliable in vitro test. However, it is difficult to collect enough extracted human anterior teeth. And the degree and chromogen of the discoloration of those teeth are varied. In the present study, artificial discolored bovine teeth (BT) as described in a previous study was used [7,10]. Extracted bovine incisors are more homogeneous than human teeth, resulting in a more standardized experimental condition. Black tea was chosen as
a high bleaching effect with an in-office bleaching material containing low concentration of hydrogen peroxide and control.

Among methods for color-measurement, the use of shade guides is subjective [14] while use of color-measuring devices is objective such as spectrophotometers or colorimeters [15]. Although the visual method can give good results in simple cases, a mechanical measurement gives better reliability when differences are not obvious [16]. For the BT experiment, the color of the specimen was measured using a colorimeter (NR-11) which was used previous studies [10-12]. And a CIE XYZ camera was used for HSP experiment. Some samples in one experimental group of the HSP study did not demonstrate uniform bleaching effect with the center of the sample showing a stronger bleaching effect. Since the CIE XYZ camera (RC500) and its equipped software can measure a small area (2 × 2 mm), the color was measured at the center of the bleached surface for the HSP experiment. This CIE XYZ camera has already been used for the color measurement of teeth and restorations in vitro [17-19].

The tooth discoloration is caused by extrinsic and/or intrinsic chromogen molecules [9]. The stains within discolored tooth are generally organic compounds that contain conjugated double bonds. The bleaching effect can occur due to the breakup of a chromophore, and that destruction of the double bonds within the conjugated system may be involved [20]. Hydrogen peroxide is widely used for in-office bleaching. The mechanism of tooth bleaching by hydrogen peroxide can be explained as follows. When an office bleaching material is applied on the tooth surface, hydrogen peroxide reacts and separates into oxygen and water molecules; $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$. During this reaction of hydrogen peroxide, several kinds of free radicals are generated [21]. Those free radicals convert the unsaturated bonds of chromogen molecules that cause tooth discoloration into saturated bonds. Those chromogen molecules in the discolored teeth are degraded to smaller, transparent, and soluble molecules, so that the stain is removed and the teeth can be bleached. However, the exact mechanism of tooth bleaching by peroxides is still not been clear [22,23].

The bleaching material used in this study (Pyrenees) contains a titanium dioxide photocatalyst. According to the manufacturer’s instruction, visible light with an intensity of more than 400 mW/cm² including light with a wavelength of 380-420 nm and an intensity of 10-130 mW/cm² are recommended. Light irradiation of the bleaching material can be expected to increase the temperature and the accelerating the reaction though the photocatalyst. The photocatalyst in Pyrenees is more reactive to lower wavelengths of light [3,24].

In the HSP experiment, the 310 nm group showed the largest bleaching effect in ΔE values. Although the 265 nm group also showed a high bleaching effect, the bleached surface was not uniform and the center of the bleached surface was whiter than other areas. In the BT experiment, 265 nm showed a statistically higher bleaching effect than 300 nm. From these results, 265 nm can be said to be the most effective for tooth bleaching. For the visible light groups, the bleaching effects of 405 and 470 nm groups in the HSP experiment and 405 + 470 nm group in the BT experiment were not enough effect due to low intensity. In this study, only low-intensity ultraviolet LED light sources were available. Clinically, a higher intensity ultraviolet LED may be necessary. The combination of a titanium dioxide photocatalyst and ultraviolet ray showed a bleaching effect without bleaching material [25]. Ultraviolet ray also enhances the decomposition of hydrogen peroxide without a photocatalyst [18]. However, ultraviolet ray shows the highest bleaching effect in the presence of both hydrogen peroxide and photocatalyst. From the results of this study, it appears that 265 nm ultraviolet ray may be most effective for the bleaching with Pyrenees.

 Ultraviolet ray can be divided into near ultraviolet and far ultraviolet rays at a wavelength of 200 nm. The near ultraviolet covers three categories; UV-A (315-380 nm), UV-B (280-315 nm), and UV-C (200-280 nm). Ultraviolet light at 365 nm, 310 (300) nm, and 265 nm belongs to UV-A, UV-B, UV-C respectively. Ultraviolet rays, especially UV-B and UV-C, are harmful to soft tissues, including oral mucosa and skin, which raises safety issues. The penetration of ultraviolet ray in the skin is wavelength-dependent. Longer wavelength (UV-A) reaches deeply into the dermis, while UV-B is completely absorbed by epidermis, likely producing cellular damage. If ultraviolet ray is applied for the tooth bleaching, teeth must be isolated to protect the soft tissues around them. Ultraviolet ray is widely used in many industries, including medical technologies. UV-A and UV-B have been subjects of research for medical applications. The phototherapy using UV-B is offered in dermatology to treat skin eruption (psoriasis), with regulatory mechanisms to minimize misuse of the treatment. UV-B irradiation induces immunosuppressive reaction, and thus narrowband UV-B might be useful for treatment of oral mucosal disorders such as periodontitis [26]. UV-A with a wavelength of 310 nm exhibited low toxicity to gingival epithelial cells [27]. Utilization of ultraviolet ray should be controlled regarding output, starting dose and dose increment [28]. For future research, a higher intensity of ultraviolet ray should be evaluated and a suitable intensity and light exposure time should be established. Further, the safety of bleaching with ultraviolet ray must be confirmed. Laboratory research must be also followed by clinical studies.

Within the limitations of the current study, it was concluded that UV-C (265 nm) and UV-B (300 and 310 nm) showed a high bleaching effect with an in-office bleaching material containing low concentration of hydrogen peroxide and
titanium dioxide photocatalyst.

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Conflicts of Interest
The authors declare that they have no conflict of interest.

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