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Application of quantitative light-induced fluorescence technology for tooth bleaching treatment and its assessment: An *in vitro* study

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ABSTRACT

Objectives: This study aimed to determine the efficacy of a combination of photocatalysts—hydrogen peroxide at a low concentration (3.5%) and titanium dioxide (TiO_2)—activated at a wavelength of 405 nm using quantitative light-induced fluorescence (QLF) technology, and to quantify their tooth-bleaching efficacy using fluorescence images obtained from QLF technology.

Materials and methods: Forty bovine incisors were extrinsically stained according to Stookey's method, and were randomly divided into four groups (n = 10 per group). Two bleaching solutions were prepared by mixing 3.5% H_2O_2 with 0.05% of anatase and rutile TiO_2 powders. These solutions were applied to the stained teeth using a microbrush and then irradiated for 15 min at either 306 or 405 nm to activate the bleaching agent. The color difference (ΔE^*) was assessed before and after every 5 min of treatment. The ΔE^* and the changes in the fluorescence loss ($\Delta\Delta F$) were obtained from white-light and fluorescence images, respectively.

Results: All of the low-H_2O_2/TiO_2 treatments caused significant tooth-bleaching efficacy after irradiation at 306 and 405 nm ($p < 0.05$). The results did not differ significantly between the two wavelengths ($p > 0.05$), but the bleaching efficacy was greater with anatase TiO_2 at 306 nm and rutile TiO_2 at 405 nm. Analysis of the fluorescence images revealed that the ΔF values increased significantly in all groups with the treatment time ($p < 0.05$). There was a statistically significant correlation between ΔE^* and the change in $\Delta\Delta F$ ($r = 0.822$, $p < 0.001$).

Conclusions: Combining low-H_2O_2/TiO_2 with QLF technology at 405 nm has an efficacy of tooth-bleaching as a less harmful and biofriendly method, while the fluorescence images obtained by QLF technology could be used to assess tooth-bleaching.

1. Introduction

Theragnosis is a new concept in which diagnosis, therapy, and monitoring are performed simultaneously using a single integrated system [1]. The emergence of this concept has resulted in interest in the medical field shifting from single-use to multifunctional devices [2]. Quantitative light-induced fluorescence (QLF) technology based on a 405 nm light source is a representative example of a multifunctional device used in dentistry. QLF technology is mainly used for diagnosing early caries and monitoring remineralization [3,4]. Recently it has been used not only for detecting cracks and bacterial deposits such as dental plaque and calculus, but also for antimicrobial treatment [5–7]. Furthermore, QLF technology can also be used as a multifunctional device in the tooth-bleaching field, reportedly being used for both increasing and evaluating the efficacy of tooth-bleaching with a 405 nm light source [8–11].

The use of a 405 nm light source helps to improve the efficacy of tooth bleaching via photocatalytic activity. Traditional in-office tooth-bleaching agents rely on highly concentrations of hydrogen peroxide (35–38% H_2O_2) to increase the efficacy of tooth bleaching. This is because the mechanism of tooth bleaching is believed to result from a complicated oxidative process involving the free radicals generated from H_2O_2 [12,13]. Even though increasing the concentration of H_2O_2 improves the efficacy of tooth bleaching, this approach is associated with side effects on the pulp [14] and the enamel surface [15]. From the viewpoint of safety, it is desirable to enhance the efficacy when using H_2O_2 at a low concentration by generating more free radicals through the application of an additional energy source such as heat or light, or by using catalysts. However, since an increase in temperature of 5.5 °C was found to cause irreversible pulp damage [16,17], it is safer to apply

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light and catalysts to accelerate the chemical reaction.

Titanium dioxide (TiO_2) is a representative photocatalytic material used in tooth bleaching, and is both nontoxic and inexpensive [18]. TiO_2 generates a strong oxidation reaction via photocatalytic oxidation when illuminated with ultraviolet (UV) light at wavelengths lower than 385 nm. However, prolonged irradiation by UV light is potentially harmful, causing adverse effects such as skin cancer [19,20]. In addition to generating UV light, other light sources such as argon, Nd:YAG diode, and CO_2 lasers are used in dentistry. In particular, 405 nm is located within the low-wavelength spectral range of visible light, which makes it safer and also appropriate for eliciting TiO_2 activity [21]. Previous studies have identified the tooth-bleaching efficacy of TiO_2 using 405 nm [10,22–24], but comparative light sources were used 470 nm LEDs or halogen lamps (400–505 nm). However, TiO_2 is generally well known to be a UV-inducible catalyst, and so it is necessary to compare the effects of light at lower wavelengths than visible light in order to confirm the bleaching effect of TiO_2 with 405 nm light.

It is also possible to evaluate the efficacy of tooth-bleaching using the natural fluorescence properties of teeth under 405 nm, since digital images are used for color analysis in the field of tooth-bleaching. The color value can be extracted from images taken under ambient light using appropriate software, while images taken during illumination at 405 nm can also be used to detect the stained areas that contrast with sound areas. Some previous studies [9,25] have identified that the fluorescence characteristics of teeth make it possible to quantitatively evaluate bleached tooth surfaces longitudinally. However, those studies used the conventional first-generation QLF device, which can only detect autofluorescence from the tooth surface. In contrast, the recently developed second-generation QLF device for use with a DSLR camera can obtain white-light and fluorescence images simultaneously, which yields various types of information from a single shot. This feature makes the second-generation QLF device suitable for use in digital imaging analysis, but to our knowledge no previous study has assessed the efficacy of tooth bleaching using this second-generation QLF device.

Previous studies of tooth-bleaching with 405 nm light only confirmed the photocatalytic effect of TiO_2 or evaluated the effectiveness of tooth bleaching using fluorescence images. In this study we determined whether QLF technology based on 405 nm light can be applied in a device for theragnosis based on not only the photocatalytic effect but also for evaluating the efficacy of tooth bleaching. Applying this to clinical practice is cost-effective since it will avoid the time associated with exchanging devices and will only require a single device to be purchased. Therefore, the first aim of this study was to determine the efficacy of a combination of photocatalysts (TiO_2 with 3.5% H_2O_2) and activation at a wavelength of 405 nm using QLF technology. The second aim was to quantify the tooth-bleaching effect by applying fluorescence imaging using QLF technology. The null hypotheses were (1) the tooth bleaching efficacy of TiO_2 with 3.5% H_2O_2 does not differ between 306 and 405 nm, and (2) there is no correlation between ΔE^* from the white-light image and the change of ΔF from the fluorescence image in the quantification of tooth bleaching efficacy.

2. Materials and methods

2.1. Preparation of enamel specimens

Forty bovine incisors were collected from a slaughterhouse and stored frozen. Teeth with cracks and discoloration were excluded from the study. All specimens were sectioned (8 mm × 4 mm × 3 mm) from 3 mm below the cementoenamel junction using a low-speed saw with a diamond blade (NTI diamond disc, NTI-Kahla, Germany). All specimens were embedded in acrylic resin and ground with 600- and 800-grit abrasive disc paper under a water-cooled polishing unit to form a flat enamel surface. Half of the surface of each specimen was covered with acid-resistant nail varnish to protect the sound tooth surface. The specimens were then stored in distilled water until testing in order to ensure that they were sufficiently hydrated.

2.2. Prestaining and extrinsic staining solution

In this study, the Stookey model, which artificially induces intense and extrinsic stain, was used to evaluate efficacy of tooth bleaching [26]. The enamel surfaces were lightly etched to promote stain accumulation and adherence before immersion in the staining solution. In the prestaining process, the specimens were first immersed in 1% HCl for 60 s, and then in supersaturated sodium carbonate for 30 s and 1% phytic acid for 60 s. The specimens were finally washed in distilled water and mounted on a custom-made staining machine for 14 days. The staining solution was prepared by dissolving 2.7 g of instant black tea, 2.7 g of finely ground instant coffee, 2.0 g of gastric mucin, and 2.0 g of ferric chloride in 800 ml of sterilized trypticase soy broth. This solution was added to 26 ml of *Sarcina lutea* broth that had been cultured for 24 h. Exogenous staining was induced that produced a brightness value of between 40 and 50, and the staining solution was changed once daily for 14 days.

2.3. Tooth bleaching process

Two bleaching solutions were prepared by mixing an aqueous solution of 3.5% H_2O_2 (Duksan Pure Chemicals, Korea) and two types of commercial TiO_2 powder at 0.05%: anatase (size < 25 nm, Sigma—Aldrich no. 637254-50 G) and rutile (size < 100 nm, Sigma—Aldrich no. 637262-25 G). A UV lamp (306 nm) or visible-light LED (405 nm) was used to activate the bleaching agent in the two groups. The power density was measured with a power meter (NOVA II). The illumination intensity was maintained at 2.1 mW/cm^2 for a spot size of 10.0 mm in order to minimize temperature effects. Each solution was applied to the stained teeth using a microbrush and irradiated by each of the light sources for 15 min. All tooth bleaching processes were performed by one single blinded examiner.

2.4. Measurement of color changes

Images of all specimen were obtained after bleaching times of 0, 5, 10, and 15 min in the absence of ambient light. White-light and fluorescence images were captured in a single shooting with a QLF technology (QLF-D Biluminator™, Inspektor Research Systems, Amsterdam, The Netherlands) under the following conditions: ISO speed of 1600, shutter speed of 1/30 s, and aperture value of 13.0 for the white-light images; and ISO speed of 1600, shutter speed of 1/20 s, and an aperture value of 7.1 for the fluorescence images. The QLF-D device has a customized DSLR camera with 4 white LEDs and 12 blue LEDs (which emit light at 405 nm).

For the first purpose of this study, tooth color difference (ΔE^*) values were measured on each of the three principal axes of the CIE $L^*a^*b^*$ color space. L^* values represent lightness and range from 0 (black) to 100 (white), while the a^* and b^* values represent the redness-to-greenness and yellowness-to-blueness axes, respectively, and ΔE^* refers to the color distance within a color space. The following formula was used to calculate color differences: $\Delta E^* = [(L^*_{\text{After bleaching}} - L^*_{\text{baseline}})^2 + (a^*_{\text{After bleaching}} - a^*_{\text{baseline}})^2 + (b^*_{\text{After bleaching}} - b^*_{\text{baseline}})^2]^{1/2}$. The CIE L^*, a^*, and b^* values from white-light images were computed using standard image analysis software (Image *J* version 1.47, National Institutes of Health, USA). The stained area was calculated within the same region of interest (ROI) by one calibrated examiner, and then ΔE^* was compared at each treatment time.

For the second study purpose, the fluorescence intensities were evaluated using the QA2 program (Inspektor Research Systems), and quantified as ΔF, which is the percentage change in fluorescence between the sound and stained areas. The ΔF was calculated with the fluorescence of sound enamel designated as 0 at the reference point, and then changes for each treatment were compared to yield $\Delta\Delta F$

Table 1
L^*, a^*, and b^* values for the different treatment groups according to evaluation period (Mean ± SD or Median (IQR)) (n = 10 in each group).

Color parameter	Groups	Tooth bleaching time			
		0min	5min	10min	15min
L^* value †	Anatase + 306 nm	46.60^{a} (45.00–47.95)	51.38^{b} (48.62–55.88)	56.26^{c} (51.81–60.29)	62.62^{d} (58.62–66.11)
	Anatase + 405 nm	47.10^{a} (44.15–50.88)	49.20^{b} (48.24–56.74)	52.88^{c} (49.47–62.52)	55.44^{d} (53.62–68.90)
	Rutile + 306 nm	46.35^{a} (44.35–50.13)	49.39^{b} (47.02–51.96)	52.42^{c} (49.56-57.88)	60.35^{d} (58.06–63.95)
	Rutile + 405 nm	47.15^{a} (43.88–49.93)	50.96^{b} (48.54–54.82)	55.60^{c} (52.08–63.23)	61.56^{d} (57.28–67.80)
a^* value ‡	Anatase + 306 nm	2.25 ± 1.12^{a}	1.84 ± 1.25^{b}	1.04 ± 1.55^{c}	0.74 ± 1.33^{c}
	Anatase + 405 nm	2.33 ± 1.14^{a}	1.32 ± 1.74^{b}	0.72 ± 1.95^{c}	0.31 ± 2.06^{c}
	Rutile + 306 nm	2.38 ± 1.74^{a}	1.80 ± 1.68^{b}	1.24 ± 1.70^{c}	0.86 ± 1.75^{c}
	Rutile + 405 nm	2.35 ± 1.56^{a}	1.74 ± 1.70^{b}	0.91 ± 2.15^{c}	0.66 ± 1.83^{c}
b^* value ‡	Anatase + 306 nm	0.93 ± 1.01^{a}	1.27 ± 1.06^{b}	1.82 ± 1.14^{c}	2.59 ± 1.18^{d}
	Anatase + 405 nm	0.80 ± 1.52^{a}	1.30 ± 1.62^{b}	1.84 ± 1.58^{c}	2.74 ± 1.24^{d}
	Rutile + 306 nm	0.65 ± 1.51^{a}	1.05 ± 1.53^{b}	1.96 ± 0.89^{c}	2.77 ± 0.99^{d}
	Rutile + 405 nm	0.68 ± 1.78^{a}	1.27 ± 1.69^{b}	2.22 ± 1.03^{c}	2.69 ± 0.91^{d}

IQR = interqurtile range.
† Different letters ($^{a-d}$) denote significant differences within or between points by Friedman's test with *post hoc* Bonferroni corrected Wilcoxon tests.
‡ Different letters ($^{a-d}$) denote significant differences within or between points by two-way repeated measures ANOVA with Bonferroni *post hoc* test.

values. The analysis was performed by copying and pasting the same stained parts as in the white-light images.

2.5. Statistical analysis

All statistical analyzes were performed with SPSS. Kolmogorov-Smirnov tests revealed that all of the a^*, b^*, ΔE^*, ΔF, and $\Delta\Delta F$ data confirmed to a normal distribution, and so parametric tests were performed. The Friedman test was used to analyze the L^* data due to the presence of large variability, and *post hoc* analysis with Wilcoxon signed-rank tests was conducted with a Bonferroni correction applied. Two-way repeated measures analysis of variance (ANOVA) was carried out to test differences between the four groups considering all treatment time and *post hoc* analysis was conducted with a Bonferroni correction applied. The correlation between ΔE^* and $\Delta\Delta F$ values used to evaluate tooth bleaching was quantified using Pearson's correlation coefficient with an alpha cutoff of < 0.05. G*Power software (version 3.1, University of Dusseldorf, Dusseldorf, Germany) was used to calculate the required sample size. Based on a statistical power, alpha level, and effect size of 0.80, 0.05, and 0.25, respectively, the specimens were randomly divided into 4 groups of 10 specimens each.

3. Results

3.1. Changes in color values (L^*, a^*, and b^*) according to treatment times

Based on the results of the repeated measures ANOVA (Table 1), a^* and b^* values showed a significant difference among the different treatment time in all groups ($p < 0.001$), but they did not differ significantly between groups at each treatment time ($p = 0.952$ and $p = 0.998$ respectively). The a^* value significantly reduced from baseline to 10 min treatment in all groups, whereas L^* and b^* value statistically significantly increased from baseline to 15 min in all groups ($p < 0.013$).

3.2. ΔE^* values for anatase and rutile TiO_2 according to 306 nm and 405 nm light sources

Table 2 lists the results for ΔE^* obtained using the two light sources with the anatase and rutile TiO_2 powders. Repeated-measures ANOVA revealed no significant differences with the type of TiO_2 or light source for each treatment time ($p = 0.563$), but significant changes were observed across treatment times ($p < 0.001$). For anatase TiO_2, the efficacy of tooth bleaching was greater at 306 nm than at 405 nm, but the differences in ΔE^* value were about 0.42, 1.55, and 3.31 after 5, 10, and 15 min, respectively. On the other hand, the difference in ΔE^* values for rutile TiO_2 showed a slightly greater bleaching effect at 405 nm than at 306 nm, with differences of about 1.34, 2.41, and 0.28 after 5, 10, and 15 min, respectively. However, for both anatase and rutile TiO_2, there were no significant differences in ΔE^* values between the two types of TiO_2 or light sources ($p > 0.05$).

Table 2
Changes in the bleaching effect (ΔE^*) for the different types of titanium dioxide (anatase and rutile) and light sources (306 and 405 nm) (n = 10 in each group).

Groups	Tooth bleaching time		
	5min	10min	15min
Anatase + 306 nm	5.02 ± 2.45^{a}	9.86 ± 3.24^{b}	15.90 ± 3.80^{c}
Anatase + 405 nm	4.60 ± 2.30^{a}	8.31 ± 4.05^{b}	12.59 ± 5.05^{c}
Rutile + 306 nm	3.15 ± 1.40^{a}	7.75 ± 4.55^{b}	14.63 ± 4.72^{c}
Rutile + 405 nm	4.49 ± 2.62^{a}	10.16 ± 5.20^{b}	14.91 ± 4.83^{c}

All values denote means ± standard deviations.
$^{a-c}$ Different superscript letters denote statistically significant differences in ΔE^* values within or between points by two-way repeated measures ANOVA with Bonferroni *post hoc* test ($p < 0.05$).

3.3. Change in the fluorescence loss after the tooth-bleaching

Fluorescence images obtained from the QLF device were analyzed using the QA2 program, and then the ΔF values after tooth bleaching were calculated. The $\Delta\Delta F$ value, which is the percentage change in ΔF, was also calculated to compare with the ΔE^* value obtained from the white-light images (Table 3). The ΔF value tended to increase with the bleaching treatment time ($p < 0.05$) regardless of the type of TiO_2 or light source. Repeated-measures ANOVA revealed no significant differences of ΔF and $\Delta\Delta F$ values among all groups ($p = 0.812$), but statistically significant changes over the bleaching time were observed ($p < 0.001$).

3.4. Correlation between color difference (ΔE^*) and the change of fluorescence loss ($\Delta\Delta F$)

Fig. 1 shows the bleaching trends in white-light and fluorescence images according to bleaching time. The bleaching trends in both types of image according to the treatment time could be identified by the naked eye. There was a strong correlation between ΔE^* and $\Delta\Delta F$ ($r = 0.822$, $p < 0.001$; Fig. 2).

Table 3
Fluorescence loss (ΔF) and its changes (ΔΔF) as measured using the quantitative light-induced fluorescence technology during the tooth-bleaching time (n = 10 in each group).

Groups	Tooth bleaching time			
	0min	5min	10min	15min
Anatase + 306 nm				
ΔF	–70.51 ± 4.27[a]	–68.42 ± 4.37[b]	–65.48 ± 5.04[c]	–62.57 ± 4.93[d]
ΔΔF		2.51 ± 1.25[a]	5.71 ± 2.01[b]	8.85 ± 2.80[c]
Anatase + 405 nm				
ΔF	–70.42 ± 7.14[a]	–68.59 ± 7.68[b]	–65.54 ± 9.41[c]	–62.91 ± 10.41[d]
ΔΔF		2.20 ± 1.55[a]	4.88 ± 2.90[b]	7.51 ± 4.01[c]
Rutile + 306 nm				
ΔF	–62.40 ± 6.94[a]	–59.61 ± 7.09[b]	–56.89 ± 6.36[c]	–54.75 ± 6.40[d]
ΔΔF		2.19 ± 0.85[a]	5.19 ± 2.45[b]	7.61 ± 2.57[c]
Rutile + 405 nm				
ΔF	–67.66 ± 8.80[a]	–65.27 ± 8.98[b]	–62.42 ± 9.27[c]	–59.12 ± 9.67[d]
ΔΔF		2.15 ± 0.95[a]	5.35 ± 2.44[b]	8.32 ± 2.83[c]

All values denote means ± standard deviations.
[a–c] Different superscript letters denote statistically significant differences within or between points by two-way repeated measures ANOVA with Bonferroni *post hoc* test.

4. Discussion

This study applied QLF technology based on a 405 nm light source—as originally used to detect oral disease—to the field of tooth-bleaching. This study has produced a less-harmful and biofriendly tooth-bleaching method based on visible light and on H_2O_2 at a low concentration, and also an objective and quantitative tooth-bleaching evaluation method using QLF technology.

The tooth-bleaching method proposed in this study aimed at increasing the bleaching effect while minimizing side effects that occur during tooth-bleaching. Therefore, a tooth-bleaching solution was prepared by mixing a TiO_2 photocatalyst with H_2O_2 at the low concentration of 3.5%, and a 405 nm light source was applied to activate the photocatalyst. Although there have been previous attempts to determine tooth-bleaching effects induced by the photocatalytic activity of TiO_2 at 405 nm, these evaluations used market products in vitro [10,22,23,27]. Most commercially available products include doped TiO_2 because this increases the effective wavelength range to increase the photocatalytic reaction by visible light. However, it has been reported that such doping treatment may decrease the photocatalytic activity due to an increase the hole–electron recombination ratio of electrons [28]. The present study therefore compared the photocatalytic activity of nanoparticles with anatase and rutile formulations of 99.9% purity. In general, the photocatalytic efficacy is reportedly higher for anatase than for rutile, but present study found no statistically significant difference between the TiO_2 crystal structures after 15 min of tooth bleaching treatment. Although the difference in the photocatalytic activity between these two major polymorphs of TiO_2 is not clear, a previous study [29] identified a difference in the generation of superoxide ions ($O_2^{\cdot -}$) and hydroxyl radicals ($HO^{\cdot}$). In the presence of H_2O_2, the amount of $HO^{\cdot}$ generated was lower for anatase than for rutile, but $O_2^{\cdot -}$ was found to be higher than anatase compared to rutile. These differences in the relative photocatalytic activities of the two formulations for generating hydroxyl radicals and superoxide ions are thought to have been responsible for no significant difference being found between the formulations in the present study.

The present study compared the tooth-bleaching efficacy between excitation by a 405 nm light source and a UV light source. This study found that the bleaching efficacy at 405 nm (visible light) was not significantly inferior to that at 306 nm (UV light) when the thermal conditions were controlled to avoid damage. This finding is similar to the results obtained by Caneppele et al [11], who performed tooth bleaching for 10 min using a combination of TiO_2 and 35% H_2O_2 and compared irradiation by an LED light source and by UV light. That study found that the ΔE^* value was 12.83 ± 5.72 for the UV light (345 nm), but 9.93 ± 6.16 for the 470 nm LED, and showed a higher bleaching effect at UV, although a statistically significant difference was not observed ($p > 0.05$). Although TiO_2 is generally known to be highly active in the presence of UV light, a nonsignificant difference was obtained between 306 and 405 nm ($p > 0.05$) since the effective wavelength can vary depending on various parameters such as the TiO_2 particle size, crystal phase, and H_2O_2 concentration.

Anatase TiO_2 with excitation at 306 nm showed the highest color difference (ΔE^*), despite there being no statistically significant differences for the various combinations of TiO_2 polymorphs and light sources (Table 2). Anatase TiO_2 has a band-gap energy of about 3.2 eV, which generates paired holes (h+) and electrons (e–) during irradiation by light at 380 nm or less, and shows a larger photocatalytic reaction. Rutile TiO_2 has a band-gap energy of about 3.0 eV and reacts strongly at 360–410 nm. The larger band-gap energy of anatase TiO_2 generally results in the photocatalytic reaction being stronger for anatase than for rutile [30]. Accordingly, the results of the present study also seem to indicate a higher bleaching efficacy for anatase TiO_2 at 306 nm than for rutile TiO_2 at 405 nm. However, this study might not have found a significant difference relative to the effect at 306 nm because the comparison was made when using a combination of H_2O_2 and TiO_2

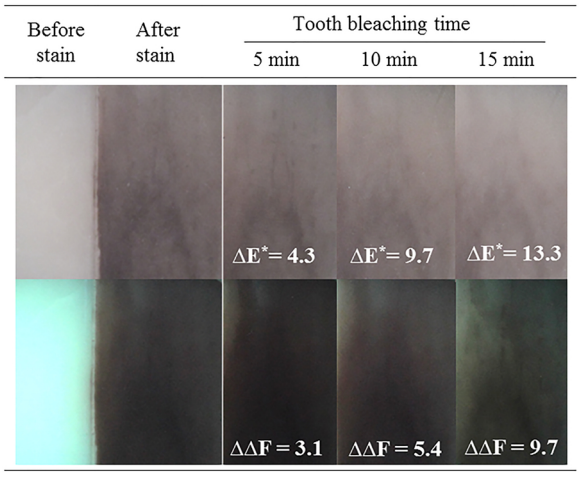

Fig. 1. Representative white-light and fluorescence images obtained using the quantitative light-induced fluorescence technology presented according to the duration of tooth bleaching for rutile titanium dioxide at 405 nm. Whiteness changes are represented by ΔE^* values and changes in the fluorescence loss are represented by $\Delta\Delta F$ values. The sound and stained parts of each specimen are on the left and right, respectively.

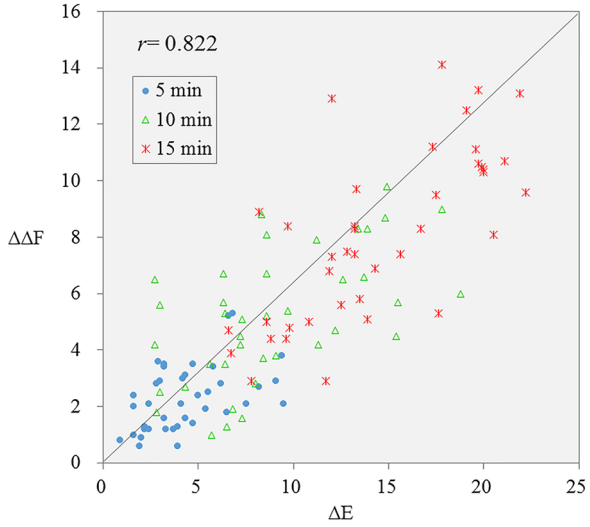

Fig. 2. Correlation between ΔE^* (whiteness changes value) and $\Delta\Delta F$ (changes in the fluorescence loss) values.

concentrations in the previous study in which the tooth bleaching efficacy at 405 nm was confirmed.

In this study, tooth bleaching was done by using the blue excitation light (405 nm) of the QLF technology. This light is a part of the QLF technology with which it is possible to quantify the autofluorescence intensity from the teeth [31]. However, the exogenous stained area showed a fluorescence with a low intensity due to the fact that stain does not transmit the blue excitation light into the underlying enamel and hence has no ability to fluoresce. After tooth bleaching, the stain was partly removed from the tooth surface allowing the blue excitation light to penetrate the enamel and the fluorescence could appear with an intensity up to that of sound enamel.

The tooth-bleaching efficacy was evaluated by comparing the change in fluorescence loss ($\Delta\Delta F$) with the more-common evaluation parameter of ΔE^*. A strong correlation was confirmed between the ΔE^* values in white-light images and the $\Delta\Delta F$ values in the corresponding fluorescence images ($r = 0.822$, $p < 0.001$; Fig. 2). This finding confirmed that it is appropriate to evaluate tooth-bleaching efficacy by using a quantitative variable obtained from the QLF technology in the future rather than making qualitative evaluations based on visual inspections under ambient light. The original ΔE^* value obtained from white-light images was inconvenient, because it has to convert the image and put in the conversion formula. In contrast, it was advantageous to calculate a quantitative value from fluorescence images obtained using the QLF technology automatically for assessing changes in tooth bleaching. Also, the second-generation QLF utilized in this study can acquire white-light and fluorescence images simultaneously. Since the first-generation QLF used in previous studies can acquire only fluorescence images, another device for evaluating tooth bleaching such as a spectrophotometer or digital camera was used to compare with the ΔE^* values obtained from the white-light images. In contrast, the QLF-D used in the present research could easily be used to calculate ΔE^* and ΔF at the same time. Thus, the second-generation QLF has the clinical advantages of providing quantitative and qualitative information simultaneously simply by taking photographs of the initial status and during the process of tooth-bleaching treatment.

This study was subject to several limitations. Firstly, it was not possible to clarify the occurrence of electron spin resonance for detecting reactive oxygen species due to the presence of photocatalytic activity. Future studies therefore also need to analyze chemical changes. Next, this in vitro study did not involve a real oral condition, such as the effects of saliva. A future in vivo study is needed to evaluate not only the efficacy of tooth bleaching when using a TiO_2 photocatalyst at 405 nm based on QLF technology, but also the possibility of assessing through fluorescence images in actual oral environmental conditions.

Notwithstanding these limitations, this research has some especially significant aspects. It is the first study to have applied the concept of theragnosis using one device in a single procedure to the field of tooth bleaching based on QLF technology. The main 405 nm light source used for QLF technology was not only used as an energy light source, but also for evaluating the tooth-bleaching efficacy using fluorescent images. In terms of clinical applications, the use of this integrated system for theragnosis is cost-effective and can reduce the chair time, and could be a safer method for tooth-bleaching.

In conclusion, this result couldn't find statistically different efficacy of tooth-bleaching between 306 nm and 405 nm while applying low energy light source. Furthermore, it was possible to quantify changes in tooth-bleaching of the stained area with fluorescence images obtained at 405 nm. We confirmed that QLF technology has the possibility of realizing theragnosis in the field of tooth-bleaching by both increasing and monitoring tooth-bleaching efficacy. This study failed to reject the first null hypothesis, since the tooth-bleaching efficacy of TiO_2 with 3.5% H_2O_2 did not differ between the 306 nm and 405 nm light sources. The other null hypothesis was rejected, with a correlation being found between ΔE^* from a white-light image and $\Delta\Delta F$ from a fluorescence image when quantifying the tooth-bleaching efficacy.

Conflict of interest

The authors declare that they have no competing interests.

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