Effect of nonthermal atmospheric discharge on tooth bleaching

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Purpose: The purpose of this study was to evaluate effect of nonthermal atmospheric discharge on tooth bleaching *in vitro* by color measurement using a dental colorimeter and an industrial colorimeter.
Materials and Methods: The air stream of nonthermal atmospheric discharge was exposed on the hematoporphyrin stained paper for 5, 10, 20, 30, and 60 minutes respectively. The air stream without atmospheric discharge (negative control) and a commercially available tooth bleaching material (positive control) were also prepared. The L*a*b* values on the treated surface at each step was measured by a dental colorimeter and an industrial colorimeter. Color difference was calculated from those values. The artificial discolored bovine teeth samples were prepared and exposed by the air stream of atmospheric discharge for 5, 10, 20, 30, and 60 minutes, and the color change was evaluated.
Results: The nonthermal atmospheric discharge showed bleaching effect for both hematoporphyrin stained paper and artificial discolored bovine teeth. Although the measured color values of both colorimeters were not consistent statistically, they showed high correlation.
Conclusion: It was concluded that nonthermal atmospheric discharge showed the bleaching effect and two colorimeters were useful for measuring color of hematoporphyrin stained paper and artificial discolored bovine teeth.

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Key Words: atmospheric discharge, color meter, plasma, tooth bleaching

Introduction

Tooth bleaching is one of the most conservative and cost-effective dental treatments to improve or enhance a person's smile and it has become one of the most popular esthetic dental treatments. There are two kinds of vital tooth bleaching treatments. One is dentist-supervised nightguard bleaching (home bleaching) and another is in-office bleaching (office bleaching). An active ingredient of tooth bleaching material is peroxides; mostly hydrogen peroxide for the office bleaching and carbamide peroxide for the home belching. To accelerate the effect of office bleaching, high concentration of hydrogen peroxide has been generally contained in the bleaching materials. Photo-activation is also employed to enhance the effect using quartz tungsten halogen lump, light emitted diode or various lasers as the light source [1]. However, the light exposure for office bleaching might not improve the bleaching effect and it increased the risk of tooth sensitivity [1].

Recently, the nonthermal atmospheric pressure plasma (NAPP) was proposed for the tooth bleaching to increase the reaction of hydrogen peroxide in the office bleaching material [2-4]. Those NAPPs were reacted with not only hydrogen peroxide but also with carbamide peroxide [5,6] and deionized water [7,8] on the tooth surface and those reactions showed tooth bleaching effect. The NAPP is generated by the atmospheric discharge. However, there has been no study on the tooth bleaching effect of the NAPP by the atmospheric discharge without peroxides or water.

The precise color measurement of the tooth is important for the evaluation of the tooth bleaching effect. Two kinds of methods were used for tooth color measurement. One is visual comparison between the target natural tooth and the tooth shade guides and another is the measurement using a color measuring devise, such as a colorimeter or a spectrophotometer. Although the conventional method using shade guides is useful and widely used, it is too subjective. Tooth color measurement using a dental colorimeter is able to obtain the objective results. For dental research concerning tooth color including tooth bleaching, the dental colorimeter and the industrial colorimeter were often used. Sometimes, it is necessary to compare the color values measured by

different colorimeters. However, there are few information about the compatibility of measured values [9].

The purpose of this study was to evaluate the effect of nonthermal atmospheric discharge (NADC) on tooth bleaching *in vitro* by color measurement using a dental colorimeter and an industrial colorimeter.

Materials and Methods

System of NADC

The experimental system of NADC used in this study was shown in Fig 1A. It was consisted from a multifunction synthesizer (Wave Factory WF1943 1CH, NF, Kawasaki, Japan), a high speed bipolar amplifier (HSA4051, NF), a digital phosphor oscilloscope (TDS 3014C, Tektronix, Tokyo, Japan), an air pump (SPP-15GA, Techno Takatsuki, Takatsuki, Japan) and a discharge tube (experimental).

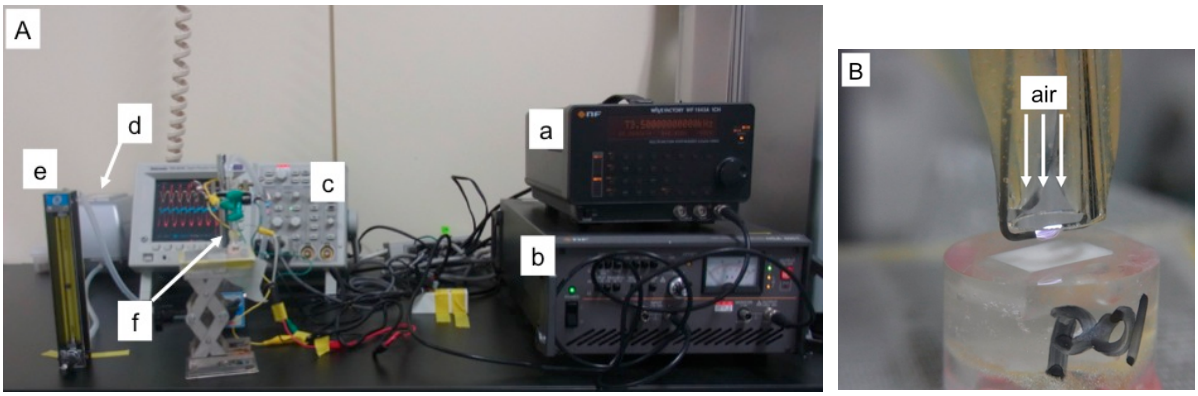


Fig. 1 Experimental apparatus
A. Overall image: a, multifunction synthesizer; b, high speed bipolar amplifier; c, digital phosphor oscilloscope, d, air pump; e, air flow meter; f, discharge tube
B. Discharge: Air flow through the discharge exposed the experimental surface

Preparation of hematoporphyrin (HP) stained paper

The hematoporphyrin (HP) stained paper was prepared by following procedure according to a previous study [10]. The 0.24 g of hematoporphyrin (Wako, Osaka, Japan) was dissolved in 300 mL of ethanol for preparing 0.1 wt% hematoporphyrin ethanol solution. The glossy white photo paper (GL-101A4100, Cannon, Tokyo, Japan) was immersed in the solution for 5 minutes and naturally dried in a darkroom. The color of the stained paper was measured by an industrial colorimeter (NR-11, NR, Nippon Denshoku, Tokyo, Japan) and the portion of L* value between 48 and 53 was chosen and employed for this study. The HP stained paper was trimmed with a suitable size with approximately 2 × 2 cm and a vinyl tape with a hole of 5 mm in diameter was put for determining the experimental area. The L*a*b* values of each HP paper sample were measured using an industrial colorimeter (NR) and a dental colorimeter (Shade eye NCC, SE, Shofu, Kyoyo, Japan).

Bleaching procedure

DC(+) group

The HP paper was placed on a flat table keeping a distance of 5 mm from the tip of a cylinder of NADC in which metal electrodes were set (Fig. 1B). Plasma was generated by discharge with a peak voltage of −5 V, a peak current value of −10 A and a frequency of 1.3 kHz. The 5 L/min of air flow through metal electrodes was exposed to surface of sample from the tip of tube (Fig. 1B). After exposure for 5, 10, 20 30, and 60 minutes, L*a*b* values of the exposed area of HP stained paper were measured using two colorimeters.

DC(−) group (negative control group)

HP paper was places on the table as well as DC(+) group. The discharge was turned off and the air stream was exposed for 5, 10, 20 30, and 60 minutes followed by color measuring using two colorimeters.

BL group (positive control group)

A commercially available office bleaching product (Pyrenees, Mitsubishi Gas Chemical, Tokyo, Japan) was used as a positive control. It contained low concentration hydrogen peroxide (approximately 3.5%) and visible light activating titanium dioxide photo catalyst. The contents of two capsules of Pyrenees were mixed well. The mixed liquid was applied on the HP stained paper and light was irradiated by a light unit (Cosmo Blue, GC, Tokyo, Japan). The light source of the light unit was violet light emitted diode (LED) with 405 nm of the peak wavelength, and the light intensity was 55 mW/cm^2. The bleaching liquid was changed at 5 minutes and every 10 minutes, and color was measured at 5, 10, 20 30, and 60 minutes.

Number of the specimen in each group was 10 (n = 10). The experimental surface of each step was also recorded by a digital camera. The color difference (ΔE) between the baseline (0 minute) and the each experimental period was calculated, according to the following equation;

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

where ΔL, Δa, and Δb are difference values of L*, a*, and b* between the baseline and each experimental period of the bleaching respectively.

Artificial discolored bovine teeth

Extracted bovine lower incisors were thawed in the running tap water. After removing soft tissues by a scalpel, the labial surface was ground by a wet silicon carbide (SiC) papers #400-1,000 to obtain flat enamel surface. Then, 5 × 5 mm specimen was prepared by a diamond cutting saw with copious water. The specimen was embedded in a dental acrylic resin (Unifast, GC) and polished by #1,200 SiC paper. The black tea was extracted from two teabags (Lipton Yellow Label, Unilever Japan, Tokyo, Japan) in 100 mL of boiled water. The samples were immersed in the tea extract for 7 days at 37˚C kept in an incubator. The solution was changed at 4th day. The color of the stained enamel surface was measured by a colorimeter (NR) and the samples of L* value between 43 and 58 were selected for this study.

The stained enamel surfaces were exposed as well as DC(+) group of the experiment using HP stained paper. The color was measured using two colorimeters at 5, 10, 20, 30, and 60 minutes. Ten specimens were prepared and evaluated (n = 10).

Statistical analysis

The L*, a*, b* and ΔE values of each group in HP stained paper experiments were statistically analyzed by two-way and one-way analysis of variance (ANOVA), then Tukey's HSD at confidential level of 0.05% (p = 0.05). The L*, a*, b* and ΔE values in bovine teeth experiments were analyzed by one-way ANOVA and Tukey's HSD. Obtained data by the dental colorimeter (NR) and the industrial colorimeter (SE) were compared by Kendall's coefficient of concordance (Kendall's W) for assessing agreement among raters and Spearman's rank correlation coefficients (Spearman's rho) for measuring rank correlation. Commercially available software (SPSS Statistics ver.21.0, IBM, Armonk, NY, USA) was used for these analyses.

Results

The typical color change of the HP stained papers in DC(+) and BL groups were shown in Fig. 2. The bleaching

effect was found in both groups. The HP stained papers in DC(−) showed no change visually. The change of L*a*b* values of each group was shown in Fig. 3.

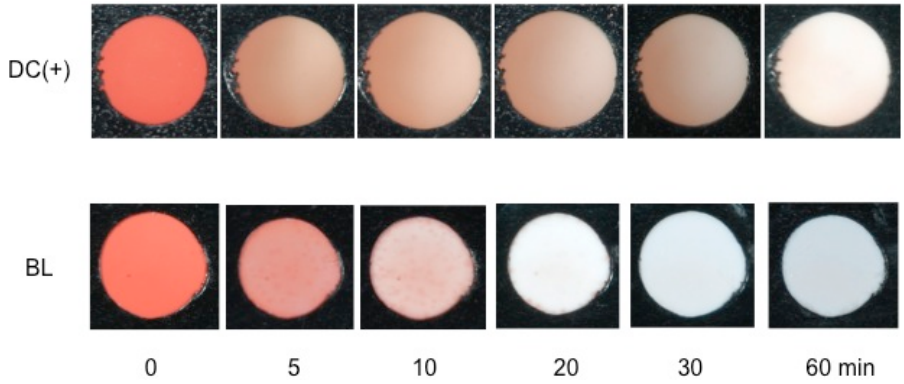


Fig. 2 Typical image of the change of HP paper in DC(+) and BL groups

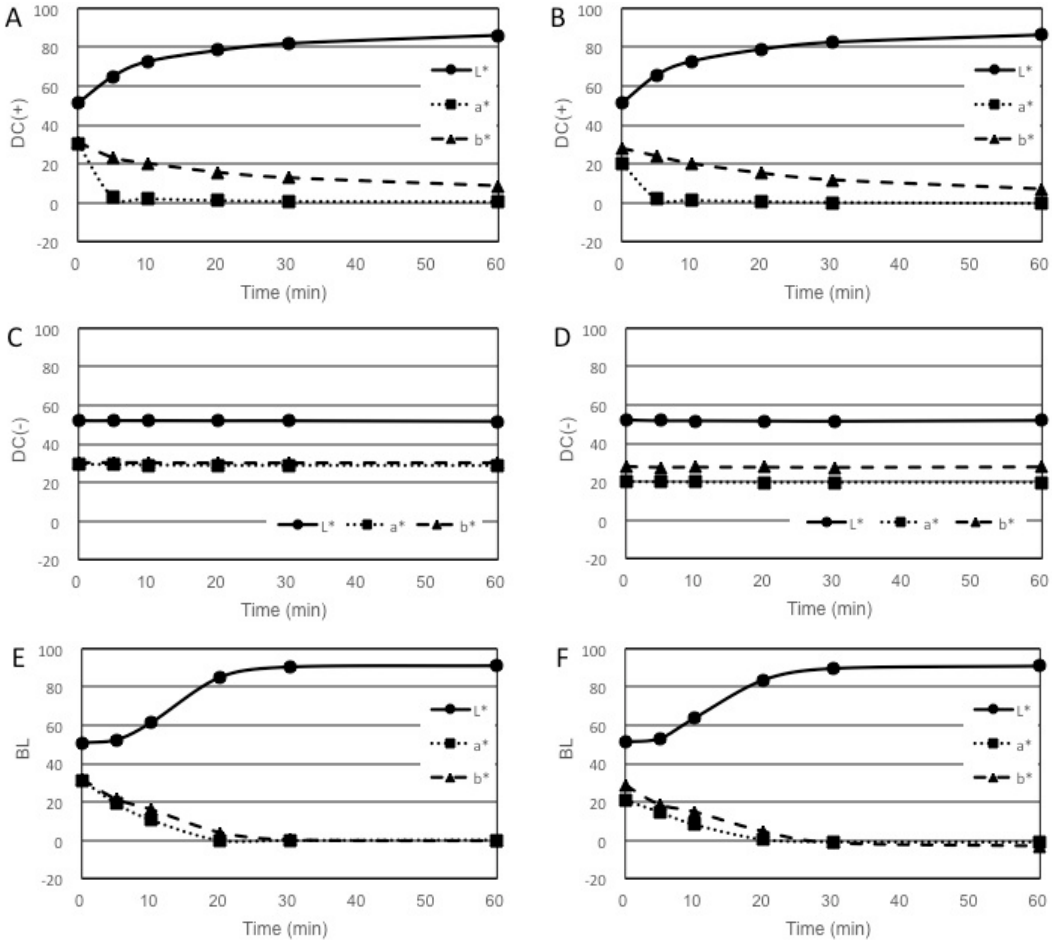


Fig. 3 Change of average L*a*b* values in each group
A, DC(+) group measured by NR; B, DC(+) group measured by SE;
C, DC(−) group measured by NR; D, DC(−) group measured by SE;
E, BL group measured by NR; F, BL group measured by SE

In DC(+) and BL groups, L* values were gradually increased and a* and b* values were decreased. For L*, a*, and b* values before exposure (time 0), there were no statistical differences among all experimental groups measured by both color meters respectively ($p > 0.05$). For L*, a*, and b* values among three groups, there were statistically differences in factor of experimental group and experimental time measured using both NR and SE

by two-way ANOVA ($p < 0.05$). The L* values in DC(+) and BL groups were much increased until 30 minutes. The L* values of DC(+) and BL groups showed statistically higher than those of DC(−) group. The a* values in DC(+) until 5 minutes and those of BL groups until 20 minutes were much decreased. The b* values in DC(+) and BL groups were much decreased until 30 minutes. The a* and b* values of BL groups showed statistically lowest followed by those of DC(+) group, then DC(−) group. For ΔE values as shown in Fig. 4, there were statistically differences in factor of experimental groups and experimental time measured using both NR and SE by two-way ANOVA. The ΔE values of BL groups showed statistically highest and followed by those of DC(+) group, then DC(−) group.

The Kendall's W of L*, a*, and b* values measured by NR and SE were 0.000, 0.886, and 0.506 respectively. Spearman's rhos of them are significant ($p < 0.05$) and were 0.980, 0.968, and 0.967 respectively.

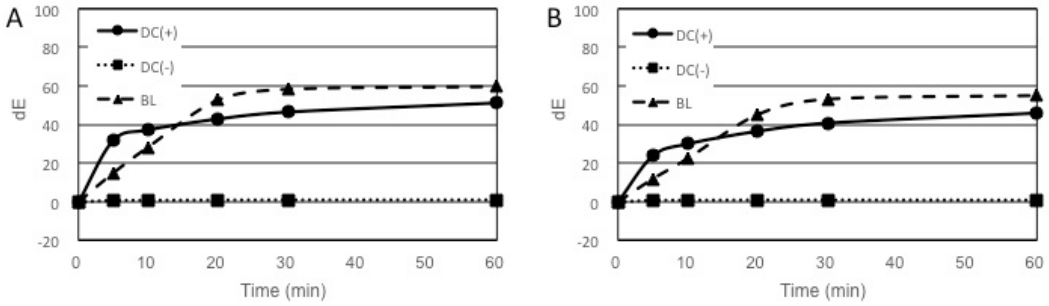


Fig. 4 The ΔE values in each experimental time A, Measured by NR; B. Measured by SE

The typical image of the change of bovine tooth by NADC was shown Fig. 5. The stain was slightly bleached by exposed time. Change of L*a*b* values of bovine tooth by DC was shown in Fig. 6. The Kendall's W of L*, a*, and b* values measured by NR and SE were 0.735, 0.308, and 0.934 respectively. Spearman's rhos of them are significant ($p < 0.05$) and were 0.949, 0.790, and 0.821 respectively. The change of ΔE values in each experimental time was shown in Fig. 7. The ΔE value was increased accompanied by increasing L* value.

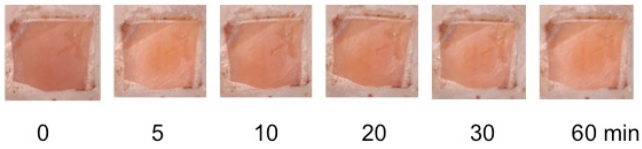
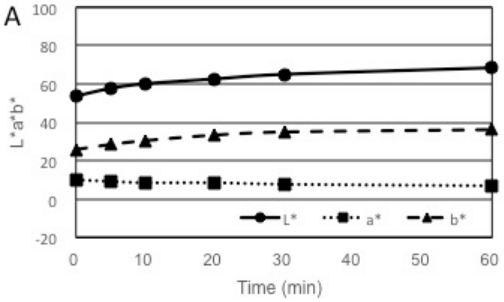
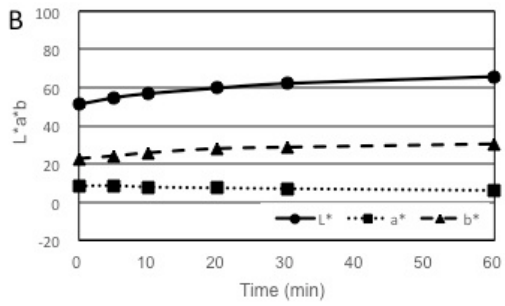


Fig. 5 Typical image of the change of bovine tooth by DC



A, Measured by NR



B. Measured by SE

Fig. 6 Change of L*a*b* average values of bovine tooth by DC

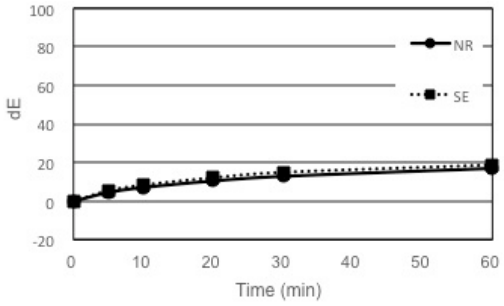


Fig. 7 The ΔE values in each experimental time

Discussion

In this study, HP stained paper and artificial discolored bovine teeth were used for evaluation of bleaching effect. This experimental method was already used for research of tooth bleaching [10,11]. The evaluation using HP stained paper is sensitive and is suitable for the screening test. However, it is difficult to predict the tooth bleaching effect from only the results of evaluation using HP stained paper. It is necessary to evaluate the bleaching effect using teeth. Since original extracted bovine teeth were very bright and whitish, they were stained by black tea extract for 7 days before the experiment. Tooth discoloration is classified as extrinsic, intrinsic, or a combination of both, and tea is one of the typical extrinsic chromogens [12]. In this study, bovine teeth were stained from both enamel surface and pulp chamber. Evaluation using artificial discolored bovine teeth can be expected to acquirer the results more clinically than using HP stained paper.

Hydrogen peroxide is widely used as an active ingredient of office bleaching. When office bleaching material is applied on the tooth surface, hydrogen peroxide in bleaching material is reacted and produces water and oxygen molecules, which reaction is accelerated by heat, catalyst and light irradiation. The concentration of HP, pH of bleaching material, application times also affect the bleaching effect. During this reaction of HP, free radicals such as oxygen ($O\cdot$), hydroxyl radical ($OH\cdot$), perhydroxyl radical ($HO_2\cdot$) and super oxide anion ($O^{2-}\cdot$) are released [13]. Tooth discoloration is caused by extrinsic and/or intrinsic chromogen molecules [12]. Those free radicals react with the chromogen molecules in the teeth and those molecules were degraded to smaller, transparent and soluble molecules, then chromogens were removed from discolored tooth and the tooth is bleached. However, the exact mechanism of tooth bleaching by peroxides has not been clear [2,4].

In this study, the plasma was generated by NADC and was contributed the bleaching effect. Plasma is the partially ionized gas [9] and the plasma technology has been widely used for industry [14]. This technology has been tried to be applied medicine including dentistry [14,15] such as surface treatments of implant [16,17], ceramics [14] and dentin [18], and polymerization of acrylic resin [19] and composite resin [20], in addition, tooth bleaching [2-7].

In the previous studies on tooth bleaching using NAPP, tooth surface was directly applied by the plasma accompanied with hydrogen peroxide, carbamide peroxide or deionized water. The chemical reaction of hydrogen peroxide and carbamide peroxide in the tooth bleaching material is accelerated by NAPP and plays bleaching effect as the mechanism above mentioned [2-5]. The bleaching effect was also shown by deionized water and NAPP [6,7]. The NAPP with water is able to produce reactive species, such as hydrogen peroxide and ozone [21,22], but also free radicals [21-23]. Those products are thought to contribute the bleaching effect.

In this study, the plasma generated by NADC was applied without peroxides or water. And the plasma by glow discharge was not directly applied on the experimental surfaces and air flow through the glow discharge was exposed. Although the mechanism of the bleaching in this study is not clear, it can be speculated that hydrogen peroxide and free radicals would be produced by plasma with oxygen and water in the air and would be delivered and reach the surface of experimental samples, then the exposed surfaces were bleached. Further study is necessary for revealing the bleaching mechanism.

The DC(+) group in HP stained paper experiment showed bleaching effect. However, this effect was less than BL group. The office bleaching material used in this study (Pyrenees) contains low concentration of hydrogen peroxide and visible light-activating titanium dioxide photo catalyst. This bleaching effect was comparable with that of another product containing higher concentration of hydrogen peroxide [10,24].

The bleaching effect in discolored bovine teeth experiment was slight and less than that in HP stained paper. Office bleaching was effective inside the dentin through the enamel [11]. It seems to be difficult to penetrate air stream and products by NADC to the dentin through enamel. The bleaching effectiveness of NADC may be limited on the enamel surface.

There are two types of color measuring devices; spectrophotometer and colorimeter. In this study, a dental colorimeter and an industrial colorimeter were used and obtained data were compared. Colorimeters measure tristimulus values and filter light in red, green and blue areas of the visible spectrum [9]. Although colorimeters is less accurate than spectrophotometers, they were widely used for both dentistry and general industry, because they are generally less expensive than spectrophotometers and still useful. The colorimeters can obtain parametric data as well as spectrophotometer, which could be easily applied for statistical analysis. ShadeEye NCC (SE) used in this study is designed for measuring the shades of natural teeth and ceramic restorations. It was reported that the shade selecting using SE could make better results than visual method for the uncomplicated cases [25].

For measuring the tooth color, it is sometimes difficult to apply an industrial colorimeter, especially for clinical situation. Although a dental colorimeter is useful for measuring tooth shade, it is not clear that a dental colorimeter can be measure the color of non-tooth samples. It was necessary to know the compatibility between a dental colorimeter and an industrial colorimeter. This study evaluated the compatibility of these colorimeters. Although the Kendall's W of a* in HP stained paper experiment and that of b* in bovine teeth experiment were high, those of L* in HP stained paper experiment and a* in bovine teeth experiment were low. Therefore, it is difficult to compare the L*, a*, and b* values measure by both colorimeters directly. Since Spearman's rho of the L*, a*, and b* values measure by both colorimeters in both experiments were very high (0.790-0.980) and statistically significant, both colorimeter can utilize for the experiments using HP stained paper and discolored bovine teeth and can evaluate the change of color by bleaching.

Tooth bleaching using peroxides is not risk-free. Tooth hypersensitivity is a major adverse effect of the tooth bleaching [13]. There is no evidence of toxic and carcinogenetic risks of hydrogen peroxide used at exposure levels of tooth bleaching [13]. However, only limited long-term clinical data are available on the side effects of tooth bleaching. Many studies reported no deleterious effects on bleached enamel and dentin surfaces concerning structure and hardness [13]. The change of temperature and morphology after bleaching by NAPP with hydrogen peroxide [2-4], carbamide peroxide [5,6], and deionized water [8] were reported and there were no deleterious effects. This study suggested the possibility of tooth bleaching by NADC technology without peroxides or water. Further studies on the safety and efficacy are necessary to establish the clinical application. It was concluded that NADC showed the bleaching effect and two colorimeters were useful for measuring color of HP stained paper and artificial discolored bovine teeth.

Conflicts of interest
None

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