Effect of nonthermal atmospheric discharge on stain removal of tooth

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The purpose of this study was to evaluate the effect of the repeated nonthermal atmospheric discharge (NADC) exposure without peroxide or water on bleaching effect of cycling stained tooth and to evaluate surface roughness and microhardness of the enamel surface exposed by NADC. Specimens with 5×5mm were prepared from extracted bovine teeth. Staining with tea and exposure by NADC were repeated for five times and color was measured at each step. Other specimens were prepared and surface roughness (Ra) and microhardness (Vickers hardness) were measured after exposure by NADC. The repeated NADC exposure without peroxide or water showed bleaching effect for stained bovine teeth. The surface roughness was not changed by NADC exposure. The less microhardness was shown by NADC exposere.

Keywords: Nonthermal atmospheric discharge, Stain removal, Microhardness, Surface roughness

INTRODUCTION

Tooth bleaching contributes each patient’s quality of life and advancement in tooth bleaching technique is quite valuable from the public health and cosmetic perspective. There are various materials used in tooth bleaching and hydrogen peroxide containing gel is most widely used. Bleaching techniques, home-based or office-based, and required treatment duration are also varied. Hydrogen peroxide is one of the most powerful bleaching agent, but its effect depends on the exposure duration and the concentration. However, hydrogen peroxide, especially high concentration one that is used in-office bleaching has been known potential of adverse effects such as hypersensitivity, enamel surface alteration and pulpal damage. Its effects and side effects have been one of major target of research.

These materials trigger oxidative reaction that breaks down the causal substances of the staining. Although the mechanism of bleaching by hydrogen peroxide is thought to be radical species production after chemical reaction, effects of these radical species on tooth enamel properties or mineral compositions were reported as limited. Bleaching effect is accelerated by heat exposure by intense light or heated instruments, however, excessive heating can cause irreversible damage to the dental pulp tissues. Therefore, many light sources have been studied to find ways to facilitate bleaching without excessive thermal exposure. In addition, photocatalytic bleaching effects of various light sources with visible light activating titanium oxide photocatalyst was proposed for effective tooth bleaching. This result implies that relatively weak light exposure could effectively trigger the sufficient level photocatalyst.

Recently, tooth bleaching by hydrogen peroxide with activation by nonthermal atmospheric pressure plasma (NAPP) has been focused because this procedure would effectively whiten the teeth without tooth surface temperature elevation that might adversely affect tooth surface property or might cause irreversible thermal pulp damage. Those NAPPs were used accompanied by not only hydrogen peroxide but also carbamide peroxide or deionized water, and those reaction showed tooth bleaching effect. The plasma was generated by nonthermal atmospheric discharge (NADC) in those study and the plasma was directly exposed to the tooth surface. It was reported that the combination of hydrogen peroxide and NAPP showed higher bleaching effect than hydrogen peroxide without NAPP. In several studies on quantifying radical production by NAPP, this facilitation of bleaching could be attributable to increased radical species production rather than deeper penetration of hydrogen peroxide.

We applied NADC on the stained tooth surface delivered by gentle air stream without existence of peroxide or water. Although the NADC alone showed the bleaching effect, this effect was less than that by a commercially available bleaching product. Since bleaching effect by NADC alone is low, repeated exposure of NADC may be effective. The purpose of this study was to evaluate the effect of the repeated NADC exposure without peroxide or water on bleaching effect of cycling stained tooth and to evaluate surface roughness and microhardness of the enamel surface exposed by NADC.

MATERIALS AND METHODS

Sample preparation

Experimental procedure was shown in Fig. 1. Freeze preserved bovine lower incisors were used for this study. They were thawed with running water. Center of labial
surfaces was trimmed by a model trimmer to obtain flat surfaces, then cut into two pieces of 5×5 mm specimens using a diamond saw (Mini Labo Cutter, Maruto, Tokyo, Japan). Each sample was embedded into acrylic tube with 10 mm in internal diameter and 10 mm of height by a self-curing acrylic resin (Unifast III Clear, GC, Tokyo, Japan). The surface of each specimen was polished by silicon carbide (SiC) papers (Fuji Star, Sankyo, Saitama, Japan) from #600 to #1200.

**Apparatus of NADC**
The experimental system of NADC used in this study was shown in Fig. 2. This generator was consisted from a multifunction synthesizer (Wave Factory WF1943A 1CH, NF, Kanagawa, Japan), a high speed bipolar amplifier (HAS 4051, NF), an oscilloscope (TDS 3014C, Tektronix, Tokyo, Japan), an air pump (High Blow SPP-15GA, Techno Takatsuki, Osaka, Japan), an acrylic tube as a discharge tube, a current probe (TCP202, Tektronix) and a voltage probe (TDP0500, Tektronix). Current voltage and frequency were adjusted by the multifunction synthesizer. A high speed bipolar amplifier enlarged voltage. Radical species were made by glow discharge and emanated from the tube. The settings of the NADC were 10 W of input electric power and 70 kHz of frequency. Air flow rate was 5 mL/min. Samples were placed on the stage keeping a distance of 5 mm between the surface of the specimen and the tube.

**Evaluation of stain removal and bleaching effect**
In the first analysis of repeated stain removal, 24 specimens were prepared and divided into 3 groups in each of eight specimens (n=8); DC+ (Discharge+), DC− (Discharge−) and Cont (Control) groups. The color of each surface was measured using a dental colorimeter (Shade Eye NCC, Shofu, Kyoto, Japan) and CIE $L^*a^*b^*$ values were obtained and photo was taken by a digital camera.

Two tea bags (Lipton Yellow Label Tea, Unilever Japan, Tokyo, Japan) were immersed into 150 mL of boiling water. The tea extract was used for the staining solution. Each sample was immersed in the staining solution for 1 min at 37°C and was treated following procedure.

- **DC+ group:** Samples were exposed by air stream from NADC apparatus for 5 min.
- **DC− group:** Samples were exposed by air stream generated by the same device without discharge.
- **Cont group (control):** Samples were placed in the constant humidity bath at 37°C, and 100% humidity for 5 min.

After those procedure, color measurement and photograph were performed. Staining and above exposure in each group were repeated 5 times; S1, S2, S3, S4, S5 and E1, E2, E3, E4, E5, respectively. The difference of $L^*$, $a^*$ and $b^*$ between each step ($\Delta L^*$, $\Delta a^*$ and $\Delta b^*$) were obtained and color difference ($\Delta E^*$) was calculated following equation.

$$\Delta E^* = (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2$$

**Surface roughness and microhardness**
In the second analysis of surface roughness and microhardness, 40 bovine incisors samples were...
prepared as well as the first experiment. The surface of each sample was further polished with SiC papers (#1500 and #2000) and diamond pastes from 6 μm up to 0.25 μm (DP-Paste P, Struers, Ballerup, Denmark). Samples were divided into 4 groups of each of 10 samples (n=10); DC+, DC−, BL (Bleaching) and Cont groups (Fig. 1).

DC+ group: Samples were exposed by NADC for 30 min with same condition of first experiment.

DC− group: Samples were exposed by air stream generated by the same device without discharge for 30 min.

BL group: Samples were bleached with an in-office bleaching material (Shofu HiLite, Shofu). The liquid and powder were mixed according to the manufacture’s instruction. Mixed paste was applied on the experimental surface for 5 min, then light was irradiated for 3 min using a quartz tungsten halogen light unit (Oputilux 501, Kerr, Orange, CA, USA). The light intensity was 520 mW/cm². After leaving for 1 min, samples were washed with running water and dried with mild air blow. This bleaching procedure was repeated 3 times.

Cont group (Control): Surface of samples were not treated.

Surface roughness was measured using a surface roughness measurement instrument (Surftest SJ-210, Mitutoyo, Kanagawa, Japan), with cutoff values of λc=0.8 and λs=2.5, and scanning speed of 0.5 mm/s. Measurement was repeated 3 times at different area in a sample. Average Ra value from 3 measurements was determined as a representative data.

The microhardness (Vickers hardness) was measured by a micro hardness tester (MVK-H2, Akashi, Kanagawa, Japan). The measuring load was 200 gf, and the measuring retention time was 15 s. Measurement was repeated 3 times at different area in a sample. Average value from 3 measurements was determined as a representative data.

Statistical analysis
The ΔL*, Δa*, and Δb* among the experimental groups were statistically analyzed by two-way and one-way ANOVA, followed by Tukey HSD test setting statistical significance at 5%. The surface roughness and microhardness were also analyzed by one-way ANOVA and Tukey HSD (p=0.05).

RESULTS
Typical color changes after first staining (S1) and fifth exposure (E5) of each group were shown in Fig. 3. Figures 4–6 showed change of L*, a* and b* values respectively at each step. After the tea staining (S1, S2, S3 S4 and S5 in Figs. 4–6), L* values were gradually decreased especially in DC+ group, and a* and b* values were increased in many stages. After exposure (E1, E2, E3, E4 and E5 in Figs. 4–6), L* values were increased, and a* and b* values were decreased in many stages. However, degree of change by treatment was different among the experimental groups. In DC+ group, increase of L* by application of NADC and decrease of L* by tea staining were high. The change of a* was small. After third-time application, change of b* was high. In DC− group, change of L* by application and tea staining showed similar pattern with those of DC+ group. However, the

Fig. 3 Typical images of samples in each group.

a. After first staining (S1) in DC+ (Discharge+) group, b. After fifth exposure (E5) in DC+ group, c. After first staining (S1) in DC− (Discharge−) group, d. After fifth exposure (E5) in DC− group, e. After first staining (S1) in Cont (Control) group, f. After fifth exposure (E5) in Cont group.

Fig. 4 Change of L* value in each stage.

DC+: Discharge+, DC−: Discharge−, Cont: Control.
amount of change was smaller than that of DC+ group. In Cont group, the change of $L^*$ was small. The changes of $a^*$ and $b^*$ in DC− and Cont groups were similar with increasing gradually.

Table 1 showed $\Delta E$ values between each step. There were statistical differences among groups ($p<0.05$). Figure 7 showed each $\Delta E$ value after the exposure in each step. There were statistical differences between DC+ and Cont groups in each stage ($p<0.05$). In S2-E2 and S5-E5 stages, statistical differences were found between DC+ and DC− groups ($p<0.05$).

Table 2 showed the surface roughness and Vickers hardness of each group. There was no significant difference in surface roughness of all groups ($p>0.05$). Vickers hardness of DC+ group showed statistically lower than those of Cont and BL groups ($p<0.05$). And there was no statistical difference in Vickers hardness between DC+ and DC− groups.

| Table 1 | $\Delta E$ values between each stage |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | S1-E1 | E1-S2 | S2-E2 | E2-S3 | S3-E3 | E3-S4 | S4-E4 | E4-S5 | S5-E5 |
| DC+             | 2.17 (1.07) | 2.55 (1.43) | 3.22 (1.21) | 2.51 (1.30) | 2.97 (1.35) | 2.39 (1.33) | 2.84 (0.97) | 2.84 (1.22) | 2.65 (0.88) |
| DC−             | 1.55 (0.83) | 1.82 (1.27) | 2.01 (0.76) | 1.66 (1.34) | 1.79 (0.78) | 2.00 (1.30) | 1.86 (1.06) | 1.25 (0.93) | 1.72 (0.83) |
| Cont            | 0.83 (0.42) | 1.50 (1.01) | 1.43 (0.61) | 1.08 (0.86) | 1.19 (0.66) | 1.28 (0.71) | 1.08 (0.95) | 1.84 (2.25) | 0.55 (0.32) |

Vertical bars showed statistical differences ($p<0.05$).

| Table 2 | Surface roughness (Ra) and Microhardness (Vickers hardness) in each group |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Surface roughness (Ra) | Microhardness (Vickers hardness) |
| DC+             | 0.059 (0.011) | 244.6 (17.6) |
| DC−             | 0.061 (0.012) | 247.2 (15.1) |
| BL              | 0.053 (0.019) | 265.4 (14.1) |
| Cont            | 0.054 (0.013) | 266.2 (17.6) |

mean (standard deviation)
Vertical bars showed statistical differences ($p<0.05$).
DISCUSSION

Peroxides have been widely used for tooth bleaching as active ingredients; mainly hydrogen peroxide for in-office bleaching and carbamide peroxide for home bleaching. The mechanism of tooth bleaching by hydrogen peroxide can be explain as follows. When an office bleaching material is applied on the tooth surface, hydrogen peroxide is reacted and is separated 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, some kinds of free radicals such as oxygen (O•), hydroxyl radical (OH•), perhydroxyl radical (HO2•) and super oxide anion (O2•−) are released\(^\text{22}\). These radical species change unsaturated bonds of organic substances that causes the tooth staining into saturated bonds. The chromogen molecules in the teeth are degraded to smaller, transparent and soluble molecules, then tooth is removed and teeth is bleached\(^\text{23}\). This reaction is accelerated by temperature, catalyst and light irradiation. The concentration of hydrogen peroxide, pH of bleaching material, and application time and times also affect the bleaching effect. However, the exact mechanism of tooth bleaching by peroxides has not been clear\(^\text{10,24}\).

In this study, the plasma was generated by NADC and gentle air flow containing this plasma contributed the stain removal of enamel surface against repeated staining. Plasma is the partially ionized gas\(^\text{25}\). The plasma technology has been widely employed for the general industry\(^\text{26}\). This technology was attempted to be applied for medical fields including dentistry\(^\text{26,27}\), such as surface treatments of implant\(^\text{28,29}\), ceramic\(^\text{28}\) and dentin\(^\text{29}\), and polymerization of acrylic resin\(^\text{31}\) and composite resin\(^\text{32}\). In addition, several studies of this technology on tooth bleaching were already reported\(^\text{13,14,15,17,19,24}\).

In many studies on tooth bleaching using NAPP, tooth surface was directly exposed by the plasma accompanied by hydrogen peroxide, carbamide peroxide or water. The chemical reaction of hydrogen peroxide and carbamide peroxide in the tooth bleaching material might be accelerated by NAPP and demonstrate bleaching effect with the mechanism above mentioned\(^\text{13,17,19,24}\). The bleaching effect was also shown by combination of deionized water and NAPP\(^\text{14,15}\). The NAPP with water is able to produce reactive species, such as hydrogen peroxide and ozone\(^\text{33,34}\), but also free radicals\(^\text{35,36}\). Those products, especially hydrogen peroxide and free radicals are thought to contribute the bleaching effect as same mechanism of tooth bleaching materials containing peroxides.

In this study, the plasma generated by NADC was applied without any peroxides or water. And the plasma by glow discharge was not directly applied on the tooth surface and air flow through the glow discharge was exposed. It was already confirmed that the tooth bleaching effect of the NADC used in this study showed tooth bleaching effect. However, this effect was less than a commercially available tooth bleaching product\(^\text{21}\). Although the mechanism of the bleaching in this study may not be clear, it can be speculated that hydrogen peroxide and/or free radicals would be produced by plasma with oxygen and water in the air and would be delivered and reached the surface of experimental samples, then the exposed surfaces were bleached. Further study is necessary for revealing the bleaching mechanism.

Extracted bovine incisors were used in the study instead of human teeth. Because enough numbers of human incisors were very difficult to collect. Bovine teeth have been used for many in vitro researches including tooth bleaching studies\(^\text{4,12,36,37}\) as they are similar to human teeth in chemical and physical properties\(^\text{38}\). In this study, an artificial discolored bovine tooth model was used, which was established in a previous study\(^\text{12}\). English tea extract was used as the staining medium similar to previous studies\(^\text{39,41}\). The stains by tea is almost entirely organic and it simulates discoloration that develops in vivo. Stains produced by tea also have no potential for calcification and they are easy to standardize, reproduce and control\(^\text{40}\).

From the first experiment of this study, DC+ group showed the stain removal effect by the exposure in each stage (E1, E2, E3, E4 and E5). The bleaching effect of NADC was less than that of a commercially available bleaching product containing hydrogen peroxide\(^\text{21}\). For discolored teeth, it may be difficult to bleach by NADC with this condition. NADC cannot be a substitution for a tooth bleaching material containing peroxides. However, this study suggested that NADC had a potential to remove stain which caused in daily life. Because NADC could remove enamel surface stain caused by repeated immersion in the tea. The tooth stain is removed by home care using tooth paste and tooth brush or by professional tooth cleaning. NADC is expected to be another option to remove stain of the teeth and to prevent accumulation of stain caused by daily foods and drinks. Further study is necessary to determine the application method and time of NADC exposure. In DC+ group, decrease of L* value was high by restaining (S2, S3, S4 and S5). By exposure of NADC, the enamel surface might be easy to adsorb the chromogen molecules of tea solution. Although the effect was less than DC+ group, L* values was increased by the exposure in DC− group. The gentle air flow from NADC would dry the surface of specimen and it might cause the increase of L* value.

The safety issues are very important for clinical use of the NADC. For the surface roughness, there was no statistically difference among all groups. However, DC+ group showed less Vickers hardness than BL and Cont groups. As there was no statistical difference between DC+ and DC− groups, gentle air stream from NADC might affect the microhardness. During production of hydrogen peroxide and free radicals by NADC with air, nitrogen in the air can be reacted by NADC. Nitrogen, hydrogen peroxide produced by NADC and water in the air can produce nitric acid and nitrous acid\(^\text{40}\). Those acids might slightly decalcify the enamel surface. For the
clinical application of NADC against the stain removal of the tooth, suitable condition of NADC exposure must be investigated and established by further studies.

CONCLUSION

With the limitation of this study, it could be concluded that the repeated NADC exposure without peroxide or water showed bleaching effect for stained bovine teeth. The surface roughness was not changed by NADC exposure. The less microhardness was shown by NADC exposure. The surface roughness was not changed by NADC exposure. The less microhardness was shown by NADC exposure. The less microhardness was shown by NADC exposure. The less microhardness was shown by NADC exposure.

CONFLICTS OF INTEREST

The authors deny any conflicts of interest related to this study.

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