

Fig. 3 (A) overall color change following nonvital tooth bleaching procedure. Non-thermal atmospheric plasma treatment significantly ($P < 0.05$) improved bleaching efficacy of common bleaching agents and deionized water. Improved bleaching efficacy could be observed visually when (B) deionized water, (C) hydrogen peroxide (D) carbamide peroxide, and (E) sodium perborate treated with non-thermal atmospheric plasma in pulp chamber.

Table 1 Color change (ΔE_3) after rehydration of teeth in artificial saliva for 2 weeks

Bleaching agent	mean $\Delta E_3 \pm SD$		P value
	without plasma	plasma	
deionized water	0.2 ± 0.09	1.8 ± 0.40	0.000*
35% hydrogen peroxide	0.5 ± 0.21	1.9 ± 0.63	0.000*
37% carbamide peroxide	0.5 ± 0.26	1.6 ± 0.73	0.000*
2:1 (w/v) sodium perborate	0.5 ± 0.14	1.6 ± 0.45	0.000*

Tooth Temperature Change

Prior to plasma treatment, the mean teeth temperatures were measured about 22°C in all experimental groups. In all NTP groups, temperature of samples was below the normal body temperature of 37°C after completion of NTP treatment. After the bleaching procedure with non-thermal atmospheric plasma, the temperature of samples increased to 30.79°C , 25.28°C , 27.86°C , and 28.02°C in HP+NTP, CP+ NTP, SP+NTP, and DIW+NTP groups, respectively, as shown in Fig. 4.

Discussion

This study was carried out not only to evaluate the enhancement of nonvital tooth bleaching efficacies of common bleaching agents when activated with NTP, but also to investigate bleaching efficacy of a safer agent—DIW—when activated with NTP to substitute it with common bleaching agents.

Hydrogen peroxide and peroxide releasing agents such as CP and SP are employed for tooth bleaching purposes

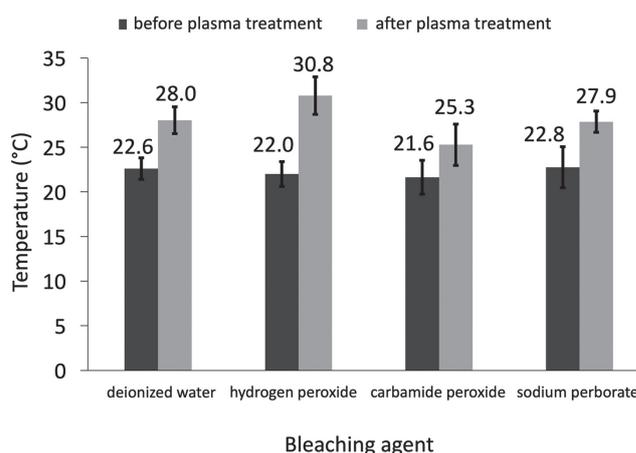


Fig. 4 Tooth temperature measurements before and after non-thermal plasma treatment. Plasma-assisted tooth bleaching procedure did not cause an increase in tooth temperature beyond normal body temperature.

in routine clinical application (1,8). However, these agents might present several risks to the tooth and might remain ineffective to achieve the desired bleaching effect (11,27). Therefore, various light and heat sources were employed to activate bleaching agents by promoting the dissociation of hydrogen peroxide to $\cdot\text{OH}$ radical (28). Nevertheless, the use of heat and/or light sources might increase the temperature in the pulp chamber above the safe limits (29). Therefore, faster and safer bleaching procedure is needed.

In this study, non-thermal atmospheric plasma treatment of common bleaching agents and DIW was limited to 5 min based on the plasma treatment time optimiza-

tion experiments. As demonstrated in Fig. 2, ΔE value reached the plateau. These results could be due to the consumption of hydrogen peroxide in 35% HP gel or limited diffusion of plasma-generated species due to loss of water from the gel after 5 min of NTP treatment.

As demonstrated in Figure 3A, NTP treatment has improved the bleaching efficacy of common bleaching agents compared to their applications. It is believed that $\cdot\text{OH}$ radical is the key radical species for tooth bleaching due to the presence of an unpaired electron, which makes it an unstable and highly reactive substance (10,30). As a result of its reactivity, $\cdot\text{OH}$ radical breaks chromophores on the tooth to smaller molecules by capturing electrons which then causes bleaching (31-33). Lee et al. has shown the release of $\cdot\text{OH}$ radical from H_2O_2 when treated with NTP using electron spin resonance technique (31). In the present study, it could be postulated that common bleaching agents were activated via plasma treatment which increases dissociation of hydrogen peroxide to $\cdot\text{OH}$ radical.

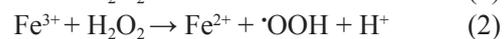
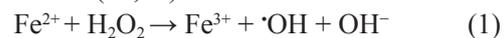
Utilization of DBD plasma electrode yielded higher ΔE values in shorter plasma treatment durations compared to previously published studies which have employed plasma jet with gas flow. The flow of the gas for generation of plasma jet removes the bleaching agent from the tooth and requires renewal of bleaching agent (22). However, the renewal of a bleaching agent is not because there was no gas flow in DBD treatment. Therefore, bleaching agents could stay in the pulp chamber during the treatment period. Thus, continuous formation and contact of chemical species responsible for bleaching with tooth could be obtained and higher bleaching efficacy could be achieved.

Despite the lack of peroxide reservoir in DIW unlike common bleaching agents, the plasma treatment of DIW in the pulp chamber also resulted in remarkable bleaching effect comparable to those achieved with plasma-activated common bleaching agents. This effect could be attributed to the formation of various ROS and RNS in plasma-treated water in the pulp chamber which makes it a reservoir of the ROS and RNS that might play roles on tooth bleaching as reported previously in several studies (22,26,34,35). Furthermore, lower consistency of water compared to common bleaching agents used in the present study, which were in the form of gel or paste, allows more efficient diffusion of plasma-generated ROS and RNS into water.

During formation of plasma discharge, various ROS and RNS are generated, and these species have been detected in several plasma-treated liquids (26,36,37). Diffusion of ROS and RNS that are generated during

plasma formation in the gas phase and reaction of these ROS and RNS with liquid causes ROS and RNS in plasma-treated liquids (38). Thus, plasma-treated liquids are considered complex mixtures of ROS and RNS, including, hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot\text{OH}$), ozone (O_3), atomic oxygen (O), superoxide (O_2^-), singlet oxygen ($^1\text{O}_2$), hydroperoxyl (HO_2), nitric oxide (NO), nitrate (NO_3^-), and nitrite (NO_2^-). Moreover, plasma treatment generates ultraviolet (UV) light (39,40). In this way, NTP treatment serves as source of various ROS and RNS that might play roles in tooth bleaching in addition to promoting dissociation of hydrogen peroxide for generation of $\cdot\text{OH}$ radical. $\cdot\text{OH}$ generation upon treatment of common bleaching agents and DIW with NTP in the pulp chamber is a result of reaction of H_2O_2 with plasma-generated species including atomic oxygen, ozone, and superoxide (41). In addition, UV light formed during plasma treatment leads to dissociation of hydrogen peroxide to form $\cdot\text{OH}$ radical (22). As previously reported by various groups, NO_3^- and NO_2^- are also generated in plasma-treated liquids, and these nitrogen species are also being thought to contribute to tooth bleaching by inducing acid pickling on tooth (22,42).

Moreover, in this study, tooth discoloration could be considered a factor for improved bleaching effect when common bleaching agents and DIW were treated with NTP. Pulpal hemorrhage is one of the main causes of internal discoloration of teeth. When blood vessels are ruptured due to trauma, blood is accumulated in the pulp chamber which leads erythrocytes to enter dentin tubules. Erythrocytes might then undergo hemolysis and hemoglobin might be liberated. Subsequently, the decomposition of hemoglobin releases iron, which then reacts with hydrogen sulfide to generate iron sulfide (Fe_2S_3), the main cause for internal tooth discoloration. In this study, teeth were stained using blood to mimic internal discoloration mechanism (23,43). Plasma-generated superoxide (O_2^-) causes release of ferrous ions (Fe^{2+}) via dissociation of iron-sulfur clusters. Liberated ferrous (Fe^{2+}) ions act as catalyst in Fenton reaction for formation of $\cdot\text{OH}$ from hydrogen peroxide via following reactions (44,45):



Color relapse due to rehydration was significantly higher in the NTP groups compared to groups in which bleaching agents were used by themselves. However, there were no statistically significant differences in the color relapse among the only bleaching agent groups or among the NTP treatment groups. The higher color relapse in the NTP-treated groups might originate from

their higher ΔE_2 values, possibly showing that the more the whitening, the more color relapse.

In this study, tooth temperature was measured before and immediately after completion of the bleaching procedure, as opposed to continuous temperature measurements in previous plasma-assisted tooth bleaching studies. As demonstrated in previous studies the tooth temperature during plasma treatment increases immediately after initiation of plasma treatment and then temperature change is equilibrated (23,31,46). Therefore, it could be postulated that by the end of the plasma treatment, teeth reached the maximum possible temperature that is induced by bleaching which was below 37°C of the body temperature. Tooth temperature exceeding 42°C leads to irreversible pulpitis and induces pulpal tissue degradation and 47°C might cause bone necrosis (14,15). The temperature increase in teeth in this study was below the normal body temperature. Therefore, activation of common bleaching agents and DIW in the pulp chamber could be considered thermally safe.

NTP treatments have been reported as non-toxic methods in various applications (47-49). However, further studies are needed to validate that plasma-generated ROS, RNS, and free radicals, which are crucial for bleaching efficacy, do not induce any toxicity in the tooth.

In conclusion, fast and effective bleaching of nonvital discolored tooth could be achieved without any thermal damage when DIW is treated with non-thermal DBD plasma in the pulp chamber. Therefore, the disadvantages of common bleaching agents could be eliminated when DIW is replaced with common bleaching agents and treated with NTP. Moreover, combination of common bleaching agents with NTP treatment in the pulp chamber leads to increased bleaching effect on nonvital tooth compared to use of common bleaching agents.

Conflict of interest

The authors have no conflict of interest to declare.

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