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Original

Deionized water can substitute common bleaching agents for nonvital tooth bleaching when treated with non-thermal atmospheric plasma

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Abstract: The bleaching efficacy of common bleaching agents and deionized water treated with non-thermal atmospheric pressure plasma in the pulp chamber for nonvital tooth bleaching was evaluated. A total of 120 extracted human maxillary first incisors were stained using human blood. Teeth were randomly divided into eight groups (n = 15). In the first four groups, teeth were bleached using 35% hydrogen peroxide gel, 37% carbamide peroxide gel, 2:1 (w/v) sodium perborate paste, and deionized water for 30 min. In the remaining groups, bleaching agents were treated with non-thermal atmospheric plasma for 5 min inside the pulp chamber. Overall color changes (ΔE) were determined using Commission Internationale de L'Eclairage Lab Colour System. The plasma-assisted tooth bleaching has not increased tooth temperature beyond 37°C. Bleaching efficacies of bleaching agents were significantly improved when treated with non-thermal atmospheric plasma compared to their application (P < 0.05). A remarkable bleaching effect was obtained when bleaching agents were substituted with water and when treated with non-thermal atmospheric plasma. Non-thermal atmospheric plasma treatment could be a novel tool for activation of bleaching agents in the pulp chamber for nonvital tooth bleaching procedure. Moreover, water could be used as a novel bleaching agent when treated with the non-thermal atmospheric plasma to eliminate possible risks which might arise from peroxide-containing agents.

Keywords: bleaching agent; blood-stained human teeth; non-thermal atmospheric pressure plasma; nonvital tooth bleaching.

Introduction

Tooth bleaching is a common aesthetic procedure in dentistry (1). Tooth discoloration is classified as intrinsic and extrinsic depending on the accumulation of chromogens either on the tooth surface or within the bulk volume of the tooth (2). Hemorrhage due to trauma, tetracycline use, infection, pulp necrosis, medications, filling materials, and restorations in the root canal are the main causes of intrinsic discoloration (3,4). Nonvital bleaching is an established clinical procedure for the elimination of intrinsic discoloration (5). Hydrogen peroxide (HP), carbamide peroxide (CP), and sodium perborate (SP) are the most common bleaching agents used for nonvital tooth bleaching (6,7). CP and SP act as a hydrogen peroxide reservoir and release hydrogen peroxide when dissociate (8). Even though the exact mechanism of tooth bleaching is not fully understood,

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one possible mechanism is the oxidation and dissolution of long-chained chromogen molecules into smaller and brighter molecules (9,10). Reactive oxygen species (ROS), in particular hydroxyl radical (•OH), released from bleaching agents, causes cleavage of double bonds in chromogens via oxidation (11,12). Light and heat application enhances tooth bleaching by accelerating the dissociation of hydrogen peroxide (13). Nevertheless, light- and/or heat-activated bleaching methods might cause an increase in tooth temperature. Consequently, the tooth temperature might exceed safe limits, which was reported as 42°C for irreversible pulpal damage and 47°C for bone necrosis (14,15). Moreover, use of hydrogen peroxide for nonvital bleaching carries potential risks such as cervical root resorption, crown fracture and worsens mechanical properties on enamel due to reduced microhardness (16). Risks originating from hydrogen peroxide could also be attributed to CP and SP as well, since they show bleaching activity through release of hydrogen peroxide from their structure (11,17-19). Thus, an effective and safe method to activate bleaching agents and a non-toxic, novel bleaching agent are needed for a safer nonvital bleaching procedure.

Plasma is the fourth state of matter and is defined as ionized gas. It is a complex mixture of free electrons, electrically excited particles, free radicals, reactive oxygen, and reactive nitrogen species (RNS), which make plasma a highly reactive medium (20). Depending on the thermal equilibrium of free electrons and ions, plasmas are classified as thermal (or hot) and non-thermal (or cold) plasmas. Non-thermal plasmas (NTP) could be applied to biological tissues for various purposes such as disinfection, coagulation, and wound healing, since it could be generated at room temperature (21). Various dental applications of non-thermal atmospheric plasmas including tooth bleaching have been reported by several groups (22-24).

In this study, a dielectric barrier discharge (DBD) air plasma has been utilized to enhance bleaching activity of common bleaching agents and to activate deionized water (DIW) for nonvital bleaching purpose as opposed to previous studies which have utilized plasma jets with a gas flow (23-25).

The aim of this study was to analyze bleaching efficacy of the most common bleaching agents when activated with direct, non-thermal atmospheric DBD air plasma treatment. Moreover, it was hypothesized that NTP treatment enhances the bleaching efficacy of common bleaching agents and thus could accelerate the entire bleaching process.

Furthermore, NTP treatment leads to the activation of various liquids including DIW via formation and diffusion of ROS, RNS and free radicals formed during plasma treatment (26). Therefore, the authors also hypothesized that NTP treatment causes activation of DIW inside the pulp chamber to induce nonvital tooth bleaching.

Materials and Methods

Plasma Source

Non-thermal atmospheric DBD air plasma was used for nonvital tooth bleaching experiments as shown in Fig. 1A and 1B. A custom-made DBD electrode was connected to a microsecond pulsed alternating current power supply (Advanced Plasma Solutions, Malvern, PA, USA) that was operated at 31 kV peak to peak voltage and 1.5 kHz frequency with 6 W power output. The DBD electrode was fabricated by covering the surface of a cylindrical copper rod with 1 mm-thick glass slide. A high-voltage cable was soldered to copper, and exposed surfaces of copper were insulated using polyethylene housing. The plasma discharge was generated by maintaining 2 mm of the discharge gap between the surface of the DBD electrode and the bleaching agent inside the pulp chamber.

Sample Preparation

The study protocol was approved by the İzmir Katip Çelebi University Non-Interventional Clinical Studies Institutional Review Board (#15, Feb 15, 2015). Human maxillary central incisors were collected in İzmir Katip Çelebi University, Faculty of Dentistry, Department of Oral and Maxillofacial Surgery. Teeth were examined under stereomicroscope (Carl Zeiss Jena GmbH, Jena, Germany) with 5× magnification. Any teeth with cracks, deformations, and/or caries were excluded and replaced with new ones. Teeth were disinfected in 10% formalin solution for 48 h. Crowns were polished using a polishing brush to remove surface staining. Endodontic access cavities were prepared using a diamond fissure and round burs under water cooling. Root canals were enlarged with a reciprocating endodontic micromotor (Reciproc, VDW, Munich, Germany), and filled with single cone gutta-percha using an epoxy resin-based root canal sealer (Meta Biomed, Cheoungju, Republic of Korea). Excess gutta-percha in the canal orifice was removed using a heated plugger. Then, the access cavities were filled with a temporary filling material (Meta Biomed, Cheoungju, Republic of Korea), and teeth were kept on humid sponges in an incubator at 37°C for 7 days.

Tooth Staining

Samples were stained using expired blood. Each sample was transferred into individual microcentrifuge tubes

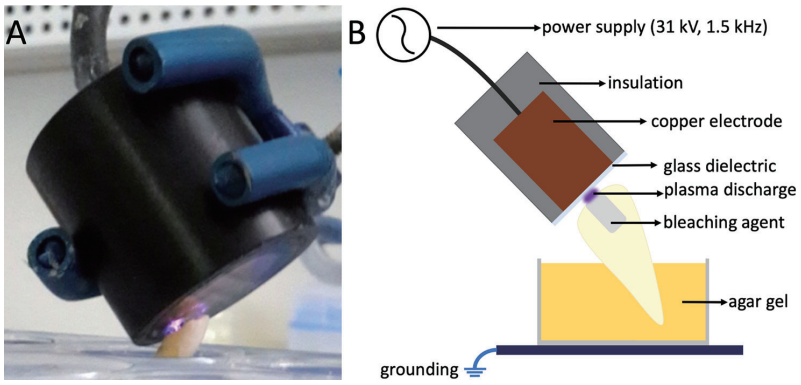

Fig. 1 Nonvital tooth-bleaching procedure by treating bleaching agents with non-thermal atmospheric plasma in pulp chamber. (**A**) Photograph of experimental bleaching procedure and (**B**) schematic diagram of plasma set-up and bleaching procedure.

containing 4 mL of blood. Samples were centrifuged at 3,400 revolutions per minute (rpm) for 20 min. Approximately 1.5 mL of plasma was removed, and samples were centrifuged for another 20 min. Centrifugation was performed twice a day for 14 days for each sample. Extracoronal staining was removed using polishing brushes in all experimental groups. Teeth with vita color of C3 or darker were included in the study.

Optimization of Plasma Treatment Time

A series of preliminary plasma bleaching experiments were carried out to determine the optimum plasma treatment time. Approximately 50 µL of 35% HP were placed in pulp chambers, and teeth were treated for 1, 3, 5, 7, and 10 min by maintaining 2 mm of the discharge gap between the surface of the DBD electrode and HP in the pulp chamber (Fig. 1A, $n = 5$). After completion of plasma treatment, bleaching agents were removed, teeth were soaked in artificial saliva and color measurements, and color change (ΔE) was plotted versus treatment time.

Tooth Color Measurement and Analysis of Bleaching Efficacy

Tooth color was measured using a dental spectrophotometer on a gray background (Spectro Shade Micro, Verona, Italy). Color measurements were repeated three times for each sample and average L^*, a^* and b^* values, which represents lightness, redness-greenness, and yellowness-blueness respectively, were recorded. Color measurements were performed before staining of samples (L_0^*, a_0^*, b_0^* values were recorded), after staining samples (L_1^*, a_1^*, b_1^* values were recorded), after bleaching experiments (L_2^*, a_2^*, b_2^* values were recorded), and 2 weeks after bleaching experiments (L_3^*, a_3^*, b_3^* values were recorded). Differences (Δ) in L^*, a^* and b^* values were determined. Overall color change (ΔE) was calculated using the following equation:

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

ΔE_1 value represents the efficacy of the staining procedure and was determined by calculating L_1^*–L_0^*, a_1^*–a_0^* and b_1^*–b_0^*. ΔE_2 value represents the efficacy of the bleaching procedure and was determined by calculating L_2^*–L_1^*, a_2^*–a_1^* and b_2^*–b_1^*. ΔE_3 value represents the color relapse in consequence of rehydration of samples in artificial saliva for 2 weeks and was determined by calculating L_3^*–L_1^*, a_3^*–a_1^*, and b_3^*–b_1^*.

Bleaching Experiments

Samples were randomly distributed to eight groups to have 15 teeth in each group. Experimental groups were determined as HP, CP, SP, and DIW (as control group) only, HP+NTP, CP+NTP, SP+NTP, and DIW+NTP. In bleaching experiments without NTP treatment, approximately 50 µL of 35% HP gel (Ultradent, South Jordan, UT, USA), 50 µL of 37% CP gel (FGM, Joinville, Brazil), and SP paste (Sigma-Aldrich, St. Louis MO, USA), which was prepared by mixing SP and DIW by 2:1 (w/v), were separately placed into pulp chambers of teeth and held for 30 min. For the control group, approximately 50 µL of DIW was placed into the pulp chamber held for 30 min. After 30 min of bleaching period, bleaching agents were removed, pulp chambers were washed with 1 mL of DIW to completely remove remnants of bleaching agents, and samples were soaked in artificial saliva solution for 5 min. Color measurements were then performed using a dental spectrophotometer on a gray background three times for each sample. In bleaching experiments in which bleaching agents and DIW were treated with NTP, similarly 50 µL of 35% HP gel, 50 µL of 37% CP gel, 2:1 (w/v) SP paste, and 50 µL of DIW were separately placed in pulp chambers, and teeth were treated with

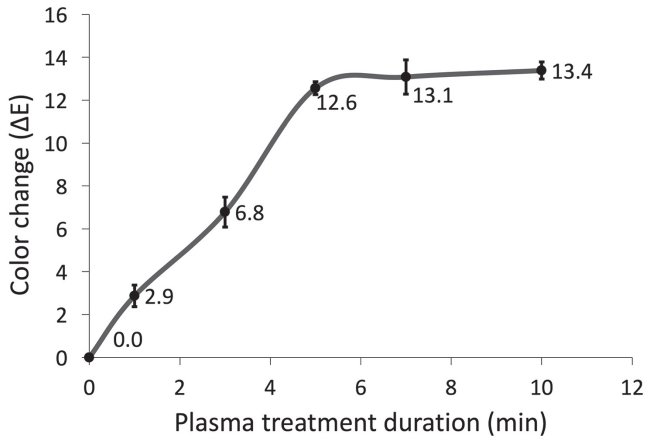

Fig. 2 Optimization of non-thermal atmospheric plasma treatment time. Hydrogen peroxide was treated for various time points in pulp chamber. ΔE value increased with increasing plasma treatment time stabilized after 5 min of plasma treatment.

non-thermal atmospheric DBD air plasma for 5 min by maintaining 2 mm of the discharge cap between the surface of the DBD electrode and the bleaching agent in the pulp chamber (Fig. 1A and 1B). After completion of plasma treatment bleaching agents were removed, pulp chambers were washed with 1 mL of DIW to completely remove remnants of bleaching agents, teeth soaked in artificial saliva, and color measurements were performed as described. Following completion of each bleaching procedure and color measurement, samples were soaked in 10% of sodium ascorbate solution to remove oxidation products and kept in artificial saliva solution at 37°C for 2 weeks. After 2 weeks, color measurements were performed to determine the stability of the bleaching procedure.

Measurement of Tooth Temperature

Temperature of teeth was measured from crown surfaces before and immediately after completion of plasma treatment using a calibrated infrared thermometer (Mesitaş, İstanbul, Turkey). Temperature measurements were performed three times before and after plasma treatment for each sample, and average temperature values were calculated.

Statistical Analysis

Statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). The normality of data distribution was tested using Shapiro-Wilk test. Color change measurements and temperature measurements were determined as parametric. Color changes were analyzed by one-way analysis of variance and a *post hoc* Bonferroni or Dunnett's *C* test (when the homogeneity of variance was <0.05). Temperature changes were analyzed by Student's *t*-test. A value of $P < 0.05$ represents statistical significance.

Results

Optimization of Plasma Treatment Time

As depicted in Fig. 2, the bleaching efficacy of the 35% HP gel when treated with NTP increased with increasing plasma treatment time until 5 min of plasma treatment. ΔE values were determined as 2.87, 6.78, and 12.56 for 1, 3, and 5 min of plasma treatment, respectively. Further plasma treatment durations did not improve the bleaching efficacy, and ΔE values reached a plateau with 13.08 and 13.38 for 7 and 10 min of the plasma treatment respectively. Therefore, plasma treatment time was determined as 5 min for further nonvital bleaching experiments.

Bleaching Efficacy

The staining procedure of the teeth caused a uniform color change in all groups with a mean ΔE_1 value of 16.07 (data not shown). There was no statistically significant difference in ΔE_1 values among groups.

Utilization of NTP treatment with bleaching agents has statistically significantly improved the efficacies of bleaching agents compared to their application ($P < 0.05$; Fig 3A). NTP treatment increased the bleaching efficacies of HP, CP, and SP by 4.72, 9.58, and 11.52 folds, respectively, compared to their use per se. Plasma treatment of DIW in the pulp chamber also led to a strong bleaching efficacy that was comparable to those obtained when common bleaching agents were treated with NTP ($P < 0.05$). The ΔE_2 values for DIW+NTP and DIW alone groups were measured as 13.19 and 0.46, respectively. Moreover, as shown in Figure 3B-E, improved bleaching efficacies of bleaching agents and DIW upon treatment with plasma could be evidently observed. The bleaching efficacies of common bleaching agents when treated with NTP did not represent any statistically significant difference ($P > 0.05$). When common bleaching agents were used, HP has shown a statistically significantly higher bleaching efficacy compared to SP and CP ($P < 0.05$).

Stability of Bleaching Efficacy

The color relapse due to rehydration was measured 2 weeks after the completion of bleaching procedures and recorded as ΔE_3. As demonstrated in Table 1, ΔE_3 values were determined as 1.94, 1.61, 1.63, and 1.79 for HP+NTP, CP+ NTP, SP+NTP, and DIW+NTP groups, respectively, and were significantly higher than those of HP, CP, SP alone, and DIW groups which were determined as 0.51, 0.49, 0.50, and 0.26, respectively ($P < 0.05$).