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An Evaluation of the Repetitive Tooth Bleaching with Nonthermal Atmospheric Pressure Plasma

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This study was undertaken to achieve a high bleaching efficacy with plasma, through longer application and reparative bleaching processes, by different shade evaluation methods. Extracted human teeth were divided into 6 groups (n=10). All teeth were treated in pairs. Low concentration of 15% carbamide peroxide (CP) was applied, with and without plasma, for 10, 20, and 30-min tooth bleaching, respectively. The bleaching procedure was repeated once daily for four days. The teeth were maintained in a moist environment provided by artificial saliva. The Vitapan Classical shade guide and Commission Internationale de L'Eclairage (CIELAB) color system were collectively used to measure the bleaching efficacy. Color evaluation was statistically analyzed using Student *t*-test and one-way analysis of variance (ANOVA) complemented by Tukey's test. Combining the plasma with 15% CP showed significantly greater color changes compared to bleaching without plasma ($p<0.05$). A high bleaching efficacy with plasma is proportional to the repetitive application and the treatment time. A 30-min application with plasma provided the best bleaching. Repetitive bleaching showed lower probability of color relapse of the bleached tooth. The color change by shade guide correlated with the changes in CIELAB color system. A value of 1 color change units (CCU) conversion factor for overall color change (ΔE) values comparisons was 3.724 values. The two measuring methods provide a more accurate correspondence of color change. The repetitive and longer application for tooth bleaching, combined with plasma, has a strong bleaching effect and produces whiter teeth.

Key words: Tooth bleaching, Nonthermal Atmospheric Pressure Plasma, Repetitive Treatment, Color Relapse

Introduction

The causes of tooth discoloration are usually various and multifaceted [1]. Tooth bleaching is an effective method to remove surface stains, and it can be accomplished by a variety of methods or systems that offer improving appearance [2].

Peroxide is the most widely used bleaching agent, in the form of hydrogen peroxide (HP; H_2O_2) or carbamide peroxide (CP; $CH_6N_2O_3$) [3]. These bleaching agents aim to treat colored organic and inorganic compounds within the dentine, thereby changing the substrate. Most of the current in-office tooth bleaching use light source or heat as the activator source to increase reaction of HP, thereby

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accelerating the decomposition of oxygen to form free radicals (HO_2,·OH) [4]. These results confirm that in-office bleaching procedure presents to reduce the total time needed to whiten teeth [5].

The successful tooth bleaching is dependent on the number and duration of bleaching sessions [6]. Especially, HP or CP concentration and contact time with tooth surfaces affect tooth bleaching response [7]. Most studies have demonstrated that the capacity of the tooth bleaching response is directly proportional to the concentration and the contact time with tooth surfaces [8,9]. A higher concentration of bleaching agents is achieved a quicker and more efficient tooth bleaching than low concentration of bleaching agents [10]. Furthermore, the tooth bleaching procedure requires repeated and prolonged application because the bleaching efficacy is expected to be greater whiten teeth and lower relapse rate [11]. In fact, clinical investigations have demonstrated that excellent bleaching results was only achieved after several treatment sessions, over a longer treatment time [12]. However, the repetitive and longer application with higher concentration of bleaching agents take the greater changes in microhardness values, inorganic/organic ratio, and the higher level of sensitivity [13]. Therefore, the low concentration of bleaching agents should be used and the repetitive treatment or contact time should be minimized for destructive effects.

Various approaches have been used to quantify the tooth color that is important for the evaluation of color change [14]. The tooth color should be measured to assess using either one of the following methods: i) special color matching scales such as porcelain shade guides; or ii) color measurement devices such as digital image analyzers. Traditionally, the shade guides used in clinical dentistry for shade taking [15]. Visual color determination with a value-ordered shade guide is a simple procedure [16]. The digital image analyzers give the ability to match color to the adjacent teeth, with sufficient accuracy to be undetectable to the critical eye. The digital image system to assess and quantify the color changes has become important [17]. Commission Internationale de L'Eclairage (CIELAB) color system has been used for the measurement of tooth color in an objective method [18], and it is capable of representing all possible colors [19].

In previous study, the treatment of nonthermal atmospheric pressure plasmaenhanced the efficacy of tooth bleaching instead of conventional light source. The application of plasma for tooth bleaching quickly decomposed HP to ·OH radical [20-23]. Tooth bleaching with plasma was highly effective at low as well as at high concentrations of HP [23]. The temperature during tooth bleaching did not increase above body temperature and maintained around 37°C. Accordingly, the plasma represents a real alternative to conventional light sources for the safe tooth bleaching by low temperature [23]. To maximize bleaching efficacy and minimum color relapse, the evaluation of repetitive bleaching with plasma and longer contact time is essential. Therefore, the purpose of this study was to evaluate the efficient and the appropriate session through repetitive tooth bleaching using nonthermal atmospheric pressure plasma with low concentration of 15% CP by different treatment time. The changes in tooth color were measured two methods using shade guide technique and CIELAB color system.

Materials and Methods

Plasma device

A microwave-driven atmospheric pressure plasma source was based on the coaxial transmission line resonator and it generated a low-temperature plasma jet (Fig. 1). The hand held microwave was operated by a palm-size power module with 2.5 W net input power. Argon gas with a flow rate of 2.5 L/min^{-1} for a safe and stable operation is used as feeding gas at atmospheric pressure in air through a flow meter (KOFLOC, Ar-05). Sufficient voltage applied to argon gas ionizes argon atoms by driving off electrons. Free electrons can trigger further ionization of adjacent to argon species by collision. This continuous reaction converts the argon gas into a partially ionized gas called plasma, which is obviously different from solid, liquid, and gas states. We covered the end of the plasma source with an acrylic cap which had a smaller output area by capillary holes of 2 mm diameter than the plasma source. By the acrylic cap, the stability of the plasma jet was increased without change of plasma characteristics, and it can be touched without feeling any thermal effect.

Tooth preparation

Thirty extracted intact human teeth were stored in 0.4% sodium azide solution at room temperature until required.

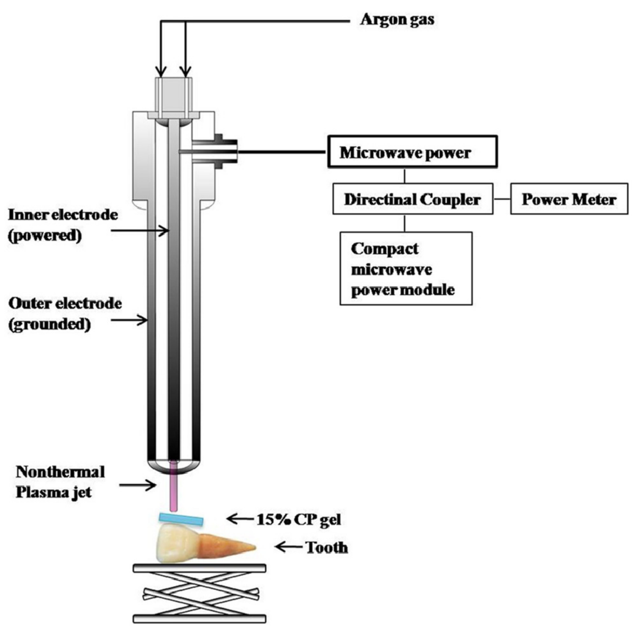

Fig. 1. Schematic drawing of the plasma device used in the study.

All teeth were thoroughly removed with an ultrasonic scaler to debris and soft tissues and polished in dental rubber cup with water/pumice slurry prophylaxis. The roots were cut with water-cooling with diamond saw (Struers Minitom, Copenhagen, Denmark) approximately 0.5 mm below the cement-enamel junction. The crown portion was cut in half longitudinally, and cut surfaces were coated with two layers of nail varnish. This study was approved from the research ethics committee of the Pusan National University Yangsan Hospital, Yangsan, Korea (approval # 04-2012-010).

Tooth bleaching procedure

Fig. 2 shows the process for overall experimental design of the study. The teeth separated into six groups, each containing ten teeth. Groups 1A and 1B was treated in pairs and applied low concentration of 15% CP, which contains 5.4% HP (Kool white 15%, Pac-dent International, USA), with and without plasma for 10 min. Groups 2A and 2B were treated in pairs with and without plasma for 20 min. Groups 3A and 3B was treated in pairs with and without plasma for 30 min. An approximately 1 mm thick of 15% CP had been covered and left undisturbed on buccal surfaces for 10, 20 and 30 min each. The tip of the plasma device was positioned at a distance of 1 cm from the surface. These bleaching procedures were repeated

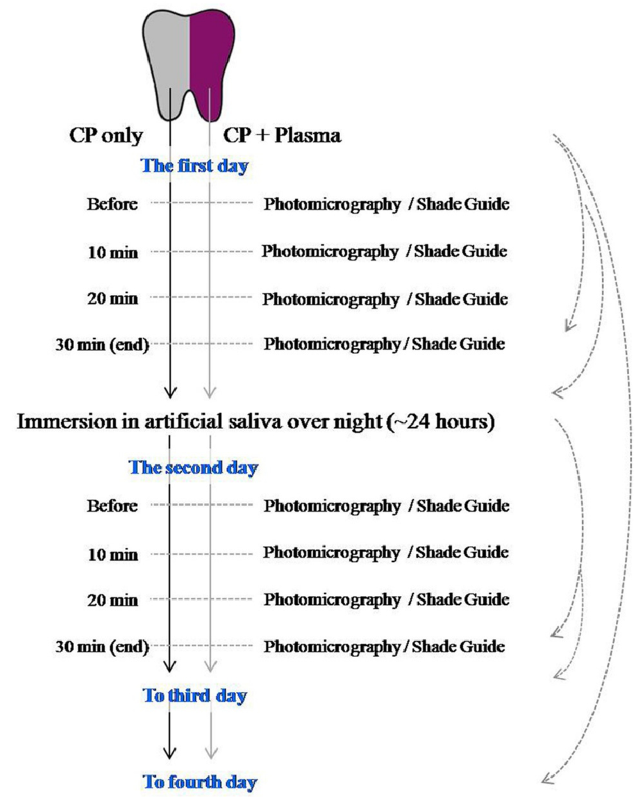

Fig. 2. Outline of the study.

four times at each treatment time, so that the total treatment time was 40, 80, and 120 min, respectively. After each application, the teeth were cleaned with distilled water, and dried with sterile gauze. And then, all teeth were subsequently immersed in individual container containing 1 mL of artificial saliva (Taliva, Hanlim, Seoul, Korea) and the artificial saliva was exchanged once every day for four days.

Shade evaluation

A color evaluation was performed using two methods to determine the color of the teeth. A comparison of color change was performed before, immediately after 10, 20, and 30 min of tooth bleaching, and color relapse after immersion in artificial from completion of the bleaching treatment. All color measurements were carried out one time every day for four days.

The shade assessment was determined using a value-orientated dental shade guide (Vitapan Classical, VITA Zahnfabrik, Bad Sackingen, Germany). Table 1 shows the standard order in which the Vitapan Classical shade guide tabs consist of 16 porcelain shade tabs. To

Table 1. The Vitapan Classical Shade Guide with 16 Shade Tabs was Arranged from Lightest (B1) to Darkest (C4) Color in Value Order.

Vitapan Classical Shade Guide															
B1	A1	B2	D2	A2	C1	C2	D4	A3	D3	B3	A3.5	B4	C3	A4	C4
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Lightest shade															Darkest shade

make determinations as accurately as possible, shade detection was always measured under the standard lighting conditions during the day in the same and the same position on a black background. And then, the buccal surface of the tooth was matched with the appropriate shade tabs and matching shade was recorded. The evaluator who was experienced in visual tooth color determination in order to avoid errors conducted all the shade comparisons and blinded to the allocation of bleached tooth regardless of the treatment.

The other method was measured digital image analyzers using the Commission Internationale de L'Eclairage (CIELAB) color system. All teeth were photographed using a digital camera (Pixel link PL-B686 CU, Ottawa, ON, Canada) fitted on stereomicroscope (SZ-CTV, Olympus, Tokyo, Japan) under standardized lighting conditions, distance and exposure, identical magnification (×10), and processed in Image-Pro Plus 5.1 software (Media cybernetics Inc., Washington, DC, USA). The images of the tooth were analyzed using a Adobe Photoshop CS2 (Adobe Systems, San Jose, CA, USA) and transformed to derive a set of numerical values. This method was performed previous plasma study also [20-23]. The L^* (black-white), a^* (green-red) and b^* (blue-yellow) values are obtained color specification within a three dimensional space. The overall color change (ΔE) is the total color difference and each group was calculated from the L^*, a^* and b^* values to compare the color changes and expressed by the following equation:

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

Two different shade evaluation methods were used to give a mean coordinate values. Distributions of ΔE values and the color change unit (CCU) were compared. The color changes were checked by correlating the mean values between two measuring methods.

Statistical analysis

Statistical analysis was performed with the SPSS computer programs (IBM Co., Version 18.0, Armonk, NY, USA). Student *t*-test was used to determine the statistical difference of color change between tooth bleaching with and without plasma at each time point. To determine the effective application sessions with the factor of reparative treatment from first bleaching, the CCU and ΔE values data were submitted to one-way analysis of variance (ANOVA) complemented by Tukey's test. The level of significance was established at a significance level of 5% ($p<0.05$).

Results

Visual evaluation by shade guide matching

The results of the CCU in shade guide assessments are shown in Fig. 3. A comparison of brightness immediately after bleaching with and without plasma is shown in Fig. 3(A). The treated tooth with plasma brightened following repetitive tooth bleaching; the mean CCU in Groups 1A, 2A, and 3A were 6.00, 7.00, and 7.60, whereas the teeth in Groups 1B, 2B, and 3B were 3.57, 4.33, and 4.38, respectively. The brightness of the CCU after the immersion in artificial saliva was shown in Fig. 3(B). The mean CCU after the immersion in artificial saliva through tooth bleaching over four repetitions in Groups 1A, 2A, and 3A was 4.83, 5.29, and 7.00, and was 1.86, 1.85, and 2.33 times brighter than Groups 1B, 2B and 3B, respectively. The repeated tooth bleaching with plasma to 10, 20, and 30 min per day for up to four times was presented significant differences for effective bleaching from 4, 3, 2 times application, respectively ($p<0.05$).

Digital evaluation by CIELAB color system

The mean ΔE values using CIELAB color system methods were shown in Fig. 4. Immediate change after 10,

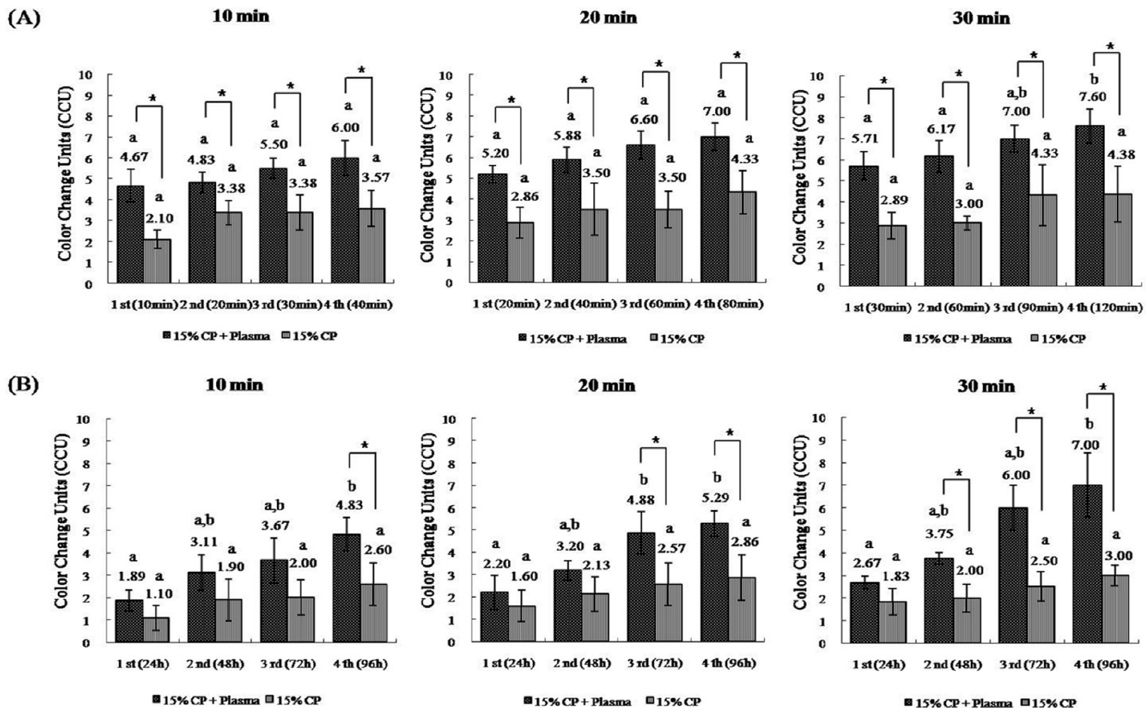

Fig. 3. Change in Vitapan Classic shade guide through reparative tooth bleaching by different treatment time. The mean color change units (ccu) of shade guide were evaluated (A) immediately after bleaching and (B) after the immersion in artificial saliva up to 4 times application.

* The statistical difference of color change between tooth bleaching with plasma and without plasma by Student *t*-test ($p < 0.05$).

a, b, c) Different letters indicate statistically significant differences by one-way ANOVA ($p < 0.05$).

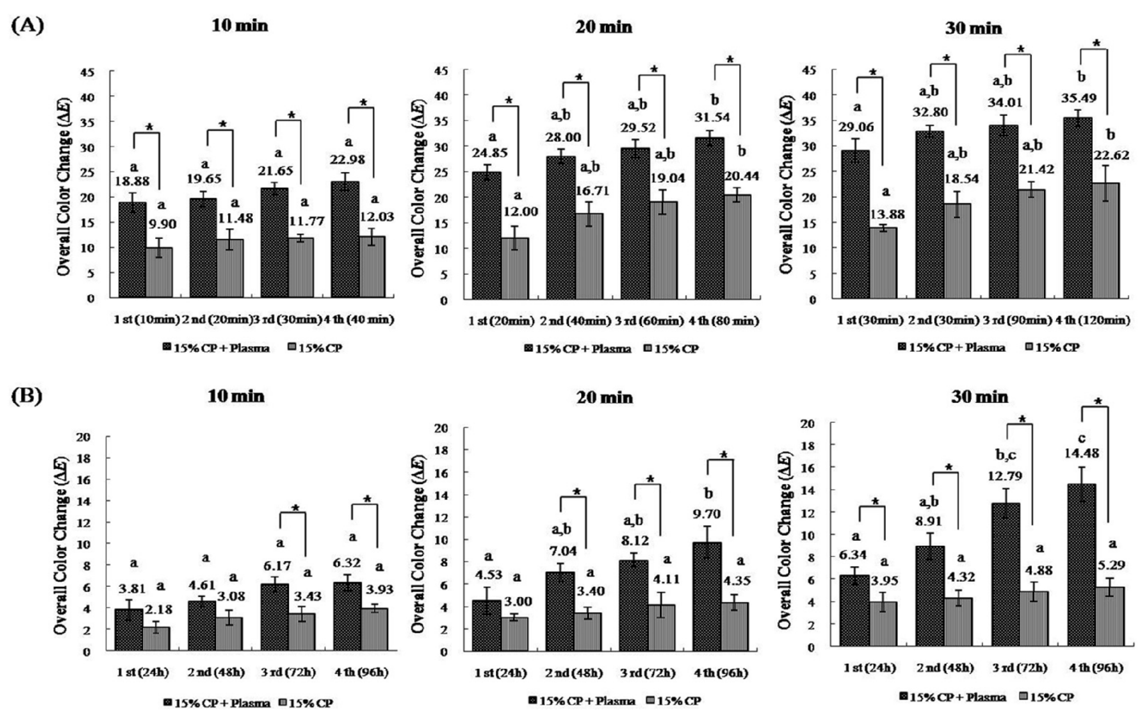

Fig. 4. The repetitive tooth bleaching effects in CIELAB color system by different treatment time. The mean values of overall color change (Δ*E*) were evaluated (A) immediately after bleaching and (B) after the immersion in artificial saliva up to 4 times application

* The statistical difference of color change between tooth bleaching with plasma and without plasma by Student *t*-test ($p < 0.05$).

a, b, c) Different letters indicate statistically significant differences by one-way ANOVA ($p < 0.05$).

20, and 30 min of reparative tooth bleaching with and without plasma was shown in Fig. 4(A). Groups 1A, 2A, and 3A showed a mean ΔE values of 22.98±1.73, 31.54±1.51, and 35.43±1.59, whereas Groups 1B, 2B and 3B showed a mean ΔE values of 12.03±1.68, 20.44±1.39, and 22.62±3.50, respectively. The mean ΔE values of Groups 1A, 2A, and 3A were significantly higher than those of Groups 1B, 2B, and 3B ($p<0.05$). The tooth bleaching in combination with plasma was significant change in brightness from the first treatment immediately after bleaching, regardless of the different time ($p<0.05$). The change in ΔE values after immersion in artificial saliva was shown in Fig. 4(B). The mean ΔE values of immersed tooth in artificial saliva after repeated tooth bleaching up to 4 times were 6.32±0.74, 9.70±1.42, and 14.48±1.57 in Groups 1A, 2A, and 3A, and were 1.61, 2.23, and 2.74 times brighter than Groups 1B, 2B, and 3B, respectively. The repeated tooth bleaching with plasma for 10, 20, and 30 min per day for up to four times was recognized significant differences for effective bleaching from 3, 2, 1 times application after treatment, respectively ($p<0.05$).

Enhancement of bleaching efficacy

As shown in Fig. 3 and 4, statistically significant difference was found depending on the reparative application and prolonged treatment time. It is presented that the bleaching efficacy with plasma is proportional to the repetitive and the longer application. Furthermore, the color changes were a higher in teeth receiving the 30 min bleaching with plasma than in teeth receiving the 10 and 20 min application. The tooth treated with plasma and low the concentration of 15% CP for the 30 min application was the best bleaching condition as the highest CCU and ΔE values. Based on these results, the reparative and the longer application of plasma may lead to faster results for enhancement of bleaching efficacy and attaining lighter shades as low color relapse than 15% CP alone.

Shade correspondence

To evaluate the relation of the visual assessment of color carried out with the Vitapan Classical shade guide and CIELAB color system. Fig. 5 showed the distribution in shade guide, together with the CIELAB color system distribution within the same diagrams as the bleaching

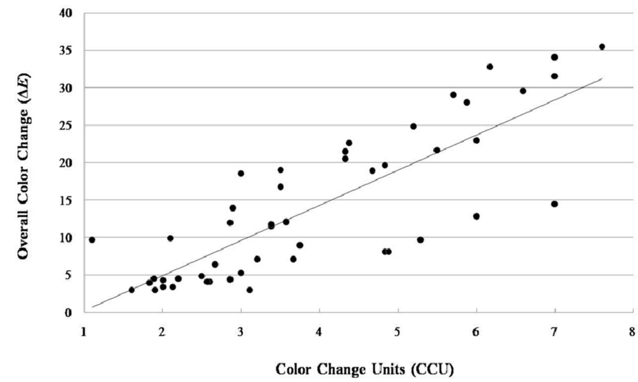

Fig. 5. Distribution of mean values regarding CIELAB color system and Vitapan Classic shade guide. The middle line is the average of the mean difference between plots indicates the corresponding values between ΔE and CCU in color coordinate values.

procedure results. The plots indicating the mean CCU and ΔE values and the middle line is the average of the mean CCU corresponding to ΔE values. An approach to 8 units in the coordinates shade guide is presented an increase in the ΔE coordinate. The present data support the relationship between CCU and the ΔE values. A 1 CCU was corresponding to 3.724 of mean ΔE values.

Discussion

Because of the continued interest in tooth bleaching, improving the materials and measurement techniques will provide better bleaching efficacy [24]. Currently, patients who require faster results cannot wait for several weeks to see the immediate esthetic improvement even after only the first appointment [25]. The bleaching effect over time should maintain the brightness. Thus, the color regression of bleached teeth remains of clinical concern.

To overcome the bleached tooth relapses, the tooth bleaching procedure requires additional applications and repeated application that is the dominant variable in determining bleaching efficacy [11]. It is known that a simple parameter is sufficient to achieve the expected bleaching outcome [12].

Joiner [14] reported that outcomes may improve with longer treatment times. Moreover, a high color changes was possible lasting bleaching effect [26]. For this reason, there are researchers who believe that the treatment must be repeated for the better results and longer contact time may influence to improve the appearance of the teeth. Therefore, the clinical demand for effective tooth bleaching grows together with the market demand for new tooth bleaching techniques. Our previous studies have resulted in tooth bleaching with plasma promotes a faster bleaching efficacy without overheating [20-23]. This study examined that is the color change of repetitive tooth bleaching in combined plasma and low concentration of 15% CP by different treatment time. A significantly a high color change was performed in 30 min applications of plasma and the reparative application was showed the decrease in the color relapses of bleached teeth. Based on these results, the repetitive application for tooth bleaching combined with plasma and low concentration 15% CP had a significant effect on improved bleaching efficacy in providing a high brightness.

Tooth shade or brightness, which is believed to be one of the most important factors in patients' perception of dental attractiveness [27]. The American Dental Association acceptance guidelines specifies color change standards for both a value-ordered shade guide and the L^*, a^*, and a^* scale [28]. In this study, the Vitapan Classical shade guide and CIELAB color system were checked together to improve the reliability to color changes. Some investigators have reported discrepancies between human-eye and digital-image analysis [29,30].

The matching shade guide is the most common method for many years as a quick verification of color choice with a value-ordered rearrangement of 16-step shade tabs [15]. Because successful bleaching indicatesfor a perceivable difference in tooth color, the human eye is a good instrument for making the types of color evaluations [29]. Accordingly, evaluation method using shade guide is clinically meaningful to both dentists and patients. However, this method is subjective and may be influenced by human physiology and physical variables such as the clinical experience, age, eye fatigue, room lighting conditions and illuminant position [31]. In addition, the range of colors available in the shade guide does not cover all possible tooth colors and it is often difficult to match a natural tooth using a shade guides [14]. Despite the reported inconsistent of color matching, shade guide is still useful in measuring the color for differentiating between dark and light colors [10].

The technology using digital camera has been used to measure tooth color and image systems have been designed to eliminate the subjectivity of color analysis [32]. Guan et al. [33] has shown that an digital image system can assess the efficacy of tooth bleaching both accurately and sensitively. The application of color science in dentistry has enabled the measurement of total color difference and tooth color quantification, with the most common color space in current use being CIELAB color system. The CIELAB color system provides more detail to distinguish minor color differences in absolute values as well as objective method, and it is capable of representing all possible colors [19]. However, some drawbacks have been described that a high cost and complex operation restricts use of the digital systems to clinical research [34]. Although there are drawbacks, it would allow an efficient method to assess the significant outcome of bleaching [18]. The ADA recommended level of color changes ≥5 CCU to indicate effective bleaching treatment [28]. Lee *et al*. [21] have reported that greater than a ΔE of 3.3 indicates an appreciable difference in dentistry.

In this study, the combinational treatment of plasma exhibits a perceptible color change by two measuring methods. After the immersion in artificial saliva at the end of four reparative processes, the 10 , 20, and the 30 min bleaching with plasma allowed 4.83 and 6.32, 5.29 and 9.70, and 7.00 and 14.48 by CCU and ΔE values, respectively. A significantly a high color change was performed in 30 min applications of plasma compared with 20 -min and 10 min, which was evident for a longer application effect. The appropriate treatment time should be performed 30 min applications by combining plasma and 15% CP, and found significant differences in CCU and ΔE values from 2 and 1 times application, respectively. This results obtained by the two evaluation methods used were a slight differentiation. The CIELAB color system exhibited more significant differences in brightness when compared with the shade guide, because it provides more sensitive to color differences as objective color parameter values. A good clinical condition should rely on the association between commercial shade guides and digital image system. In this study, shade guide is the simultaneous comparison of the color change with the CIELAB color system. Two measuring method is possible to interplay the results obtained between CCU and ΔE values, and it provides a broader perspective and a more accurate correspondence of color change that can be expected. A 1 CCU, the conversion factor was perceived a color difference of 3.724 ΔE values.

Furthermore, the repetitive and longer application for tooth bleaching combined with plasma and low concentration 15% CP had a significant effect on improved bleaching efficacy and minimized color relapse in providing a high brightness.

At the time of establishing a protocol for the use of plasma for tooth bleaching, this approach would provide us with preliminary results before expensive clinical trials and would lead to appropriate treatment time and repetitive application sessions for tooth bleaching. Therefore, tooth bleaching technique combined with plasma shows reasonable promise of being practical in the future.

Conclusion

The repetitive and longer application for tooth bleaching combined with plasma had a significant effect on improved bleaching efficacy in providing a high brightness and low color relapse. Two methods with Vitapan Classical shade guide and CIELAB color system was presented the efficient interaction in determining the color change of tooth bleaching. These combined data are more useful because it provides a more accurate estimate of the degree of color change.

Acknowledgements

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Conflict of interest

The authors declare that they have no competing interest.

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