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Assessment of Structural and Compositional Safety of Dentin Treated with Water-Based Cold Plasma (Magnetic Fields)

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Recently, a growing demand for new bleaching techniques that minimize potential damage to dental hard tissues while maintaining bleaching efficacy has been observed in clinical practice. Physically-activated energy-based bleaching methods that assist or replace chemical oxidation reactions are attracting attention. This study evaluated the effects of using sterile distilled water (DW) and cold plasma-based bleaching for vital bleaching (bleaching of vital teeth) on the internal dentin structure. Additionally, the study verified whether the structural stability of dentin hard tissue was maintained after bleaching. Dentin specimens, measuring 1 mm × 2 mm × 1 mm, were prepared and randomly assigned to four groups, untreated control, each receiving either sterile DW or cold plasma alone or in combination for 30 minutes (n = 10/group). Cold plasma treatment was driven by argon (Ar) gas at approximately 3 kVpp and 20 kHz, and irradiated from a distance of 1 cm from the dentin surface. In the plasma-based group, sterile DW was applied, and then reapplied to the specimen surface at 30-second intervals, while plasma was simultaneously irradiated. After all treatments, the specimens were washed with DW and dried. The microhardness was measured using a Vickers hardness tester, and the changes in mineral compositions were determined by comparing the weight ratios of major elements through electron probe microanalysis (EPMA). After 30 minutes of treatment, no significant changes were observed in the dentin surface microhardness and mineral composition in any of the experimental groups ($p > 0.05$). The study results demonstrated that the single or combined application of sterile DW and/or cold plasma did not affect the dentin hard tissue. These results suggest that bleaching of vital teeth using sterile DW and cold plasma does not induce significant changes in the microhardness and mineral composition of dentin. As such, sterile DW and cold plasma show promise for hard tissue-preserving bleaching applications in esthetic dentistry.

Keywords : cold atmospheric plasma, distilled water, microhardness, mineral composition

1. Introduction

Bright white teeth play a crucial role in aesthetics. As the expectations for esthetic improvement grow, so does the demand for tooth bleaching treatments [1]. Tooth bleaching is a procedure that restores teeth to their original color or brightens them even further using only chemicals without removing any dental plaque [2]. High-concentration hydrogen peroxide (H_2O_2), a powerful oxidizing agent, produces highly reactive free radicals during its decomposition process, oxidizing and decomposing stains and organic compounds on the tooth surface, resulting in a bleaching effect [3]. Additionally, light irradiation can accelerate the bleaching process if necessary. Light energy activates the penetration of H_2O_2 molecules into the numerous microscopic pores of the teeth, resulting in rapid bleaching of the teeth [4].

H_2O_2 the main ingredient in tooth bleaching products, is a strong oxidizing agent. At high concentrations, H_2O_2 can damage tooth hard tissues, causing increased tooth sensitivity, gum burns, and external resorption of the cervical region. It can also cause damage or corrosion of existing restorations [5]. Furthermore, as H_2O_2 molecules are small, they can easily reach the dental pulp through the dentinal tubules, irritating nerve endings, and causing

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hypersensitivity [6]. High concentrations of 30-35% H_2O_2 can cause mucosal burns and gingival discoloration. Animal studies have shown that applying 1% H_2O_2 to the gums for 6-48 hours can cause epidermal damage and acute inflammation of the subepidermal connective tissue [7].

In dentistry, tooth bleaching techniques that safely improve tooth color and enhance esthetics are sought after. Consequently, bleaching products and technologies are rapidly developing [8]. Recently, cold plasma applications have been expanding into diverse fields, including medical and environmental applications. This expansion is attributed to cold plasma's ability to efficiently generate highly chemically reactive species while maintaining a relatively low gas temperature. In dentistry, cold plasma has been shown to induce tooth bleaching by promoting the production of hydroxyl radicals [9]. When applying cold plasma, the temperature inside the pulp chamber is maintained at 37 °C, and temperature increase is controlled [10]. Cold plasma maintains the tooth surface temperature at approximately 37 °C, which is within the physiological temperature range, during the bleaching process, and is therefore considered a safe light source with low heat transfer and low risk of thermal damage [11]. In contrast, the light source used in conventional light-activated tooth bleaching can raise the tooth surface temperature to approximately 43 °C, increasing the risk of damage to the pulp and surrounding tissues. Histological stability has been proven through animal testing, and no inflammatory reactions or structural damages have been observed in soft tissues, such as the pulp, gums, or oral mucosa, when tooth bleaching using cold plasma, confirming that it is a safe and effective energy source that can replace existing light sources [12]. Although the effect of bleaching dentures using a light source has been studied [10], a systematic evaluation of the structural stability of dentin directly in contact with cold plasma during denture bleaching has not been reported. Therefore, this study aimed to verify the structural stability of dental hard tissues and inside the pulp chamber during the bleaching process using distilled water (DW) and cold plasma instead of high-concentration H_2O_2 during denture bleaching.

2. Materials and Methods

2.1. Cold atmospheric-pressure plasma device

Plasma is generated in the handpiece, which contains a coaxial dielectric barrier discharge (DBD) source composed of a stainless steel (SS 306) inner electrode and an outer electrode surrounding a ceramic nozzle (Al_2O_3). Activation of the main unit initiates argon (Ar) flow through the space between the electrodes, while a 3 kVpp alternating voltage at 20 kHz is applied to generate the plasma. The main unit also houses a switched-mode power supply (SMPS), a solenoid valve, a gas flow regulator, and high-voltage circuitry. The Ar flow rate was maintained at 1 slm (standard liter per minute) via the flow controller. Plasma was generated under a magnetic field condition; however, the magnetic field itself was not controlled or evaluated as an independent variable in this study. The cold plasma operating device employed in this study is shown in Fig. 1.

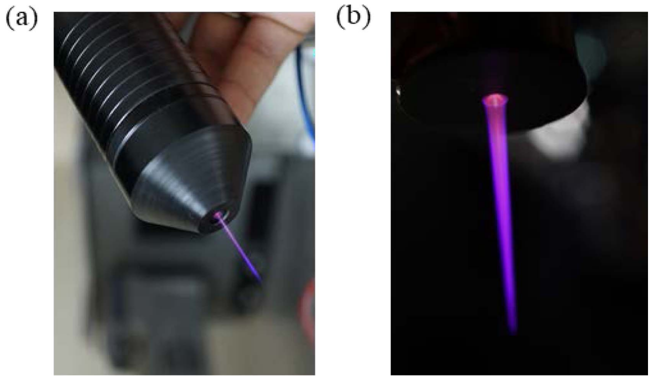

Fig. 1. (Color online) Cold atmospheric-pressure plasma device used for tooth bleaching. (a) Pen-type plasma device operated with argon (Ar) gas. (b) Generation of ionized plasma plume under magnetic field conditions during application.

The governing equations for the non-isothermal gas flow, including the plasma fluid model, continuity, drift-diffusion fluxes, electron energy balance, and Poisson's equation for the electric field, have been described in detail in previous studies [13].

2.2. Preparation of specimens and tooth bleaching procedure

A total of forty extracted human teeth, free of caries, cracks, fractures, and erosions, were selected for this study. The use of human teeth was approved by the Ethics Committee of the Dental Hospital of Pusan National University (PNUDH-2014-025). Each tooth was sectioned at the cementoenamel junction using a low-speed saw (Minitom, Struers, Copenhagen, Denmark), and thoroughly cleaned with an ultrasonic scaler to remove calculus and periodontal remnants. The dentin portions of the teeth were sectioned and prepared as 1 mm × 2 mm × 1 mm slabs for analysis. The specimens were then randomly assigned to four groups.

The prepared teeth specimens were randomly allocated into four groups (n = 10 per group). The specimens in

Group 1 served as the untreated control and were stored in artificial saliva without any additional surface treatment. Group 2 underwent treatment with cold atmospheric plasma for 30 minutes. In Group 3, the specimens were immersed in DW for 30 minutes. In Group 4, the specimens were treated with DW at 30-second intervals while cold atmospheric plasma was simultaneously applied, maintaining a constant distance of 1 cm between the device tip and the dentin surface throughout the 30-minute treatment period. After completing the respective treatments, all specimens were rinsed with DW and air-dried.

2.3. Microhardness measurement

The microhardness of the dentin specimens was measured using a Vickers hardness tester (Akashi MWK-III, Tokyo, Japan). A load of 100 g was applied perpendicular to the dentin surface for 10 s, and the Vickers Hardness Number (VHN) was recorded. Measurements were taken at four points per specimen under 400× magnification, and the mean and standard deviation values were calculated for each group (n = 10).

2.4. Elemental composition analysis

The elemental composition of the enamel and dentin surfaces was analyzed using an electron probe micro-analyzer (EPMA; SX100, CAMECA, Corbevoie, France) to quantify changes after the 30-minute application in each group (n = 10). The specimens were coated with carbon prior to the analysis, and examined under the following operating conditions: 10-μm probe diameter, 15-keV accelerating voltage, and 20-nA beam current. Elemental concentrations of magnesium (Mg), phosphorus (P), chlorine (Cl), calcium (Ca), zinc (Zn), and sodium (Na) were measured at four points of each specimen, and the mean weight percentage values were calculated for each group.

2.5. Statistical analysis

The means and standard deviations of all the specimens in each group were calculated. The difference in groups were analyzed one-way analysis of variance (ANOVA) and a post hoc Tukey's test with a 95% significance level, using SPSS, Version 24 (SPSS, Chicago, IL, USA).

3. Results

3.1. Change in microhardness value

The dentin surface microhardness was assessed following the 30-minute treatments. As shown in Fig. 2, no statistically significant differences in VHN were observed among the groups ($p > 0.05$), indicating that cold plasma application treatment did not affect the surface microhardness of dentin.

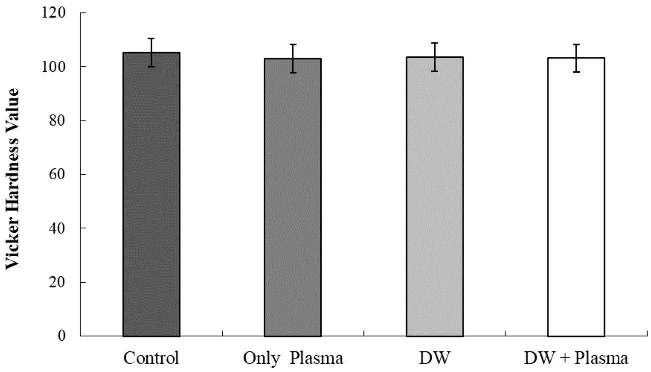

Fig. 2. (Color online) Surface microhardness of the dentin after a 30-min treatment, expressed as mean ± standard deviation (SD).

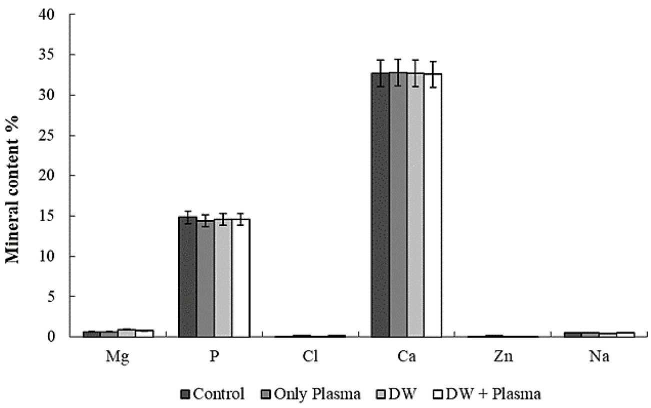

Fig. 3. (Color online) Magnesium (Mg), phosphorus (P), chlorine (Cl), calcium (Ca), zinc (Zn), and sodium (Na) levels in dentin surfaces after 30 minutes of treatment, presented as mean ± standard deviation (SD).

3.2. Changes in mineral contents

Mg, P, Cl, Ca, Zn, and Na levels in the enamel are presented in Fig. 3. No significant differences were observed among the treatment groups following treatment. ANOVA confirmed that the mineral composition of the enamel remained unchanged across all groups ($p > 0.05$), indicating that cold plasma treatment in DW application did not affect the elements.

4. Discussion

Social interest in bright white teeth and the demand for tooth bleaching, which brightens the color of teeth, have increased, leading to a rapid advancement in the clinical use of tooth bleaching techniques [14].

The causes of tooth discoloration can be broadly divided into congenital and acquired causes. Congenital causes include maternal exposure to antibiotics, such as tetracycline or excessive fluoride intake. Acquired causes arise from changes in the tooth's life history, including pulpal bleeding, pulp necrosis due to trauma, staining caused by food, tobacco, coffee, cola, and other palatable foods and beverages, as well as age-related discoloration [15]. In non-vital teeth, hemoglobin breakdown products and protein degradation products generated during pulp necrosis or root canal treatment can penetrate the dentinal tubules, causing internal discolorations [16]. Internal discolorations are difficult to improve with physical bleaching means alone because physical bleaching targets the external surfaces. Instead, specific bleaching techniques are used to address internal discolorations. These methods involve injecting a bleaching agent into the pulp or upper root canal space to oxidize and decompose pigments within the dentin [17]. High-concentration H_2O_2 or sodium perborate are two common agents used for vital and non-vital bleaching of teeth. However, these oxidizing agents can induce dentin demineralization and changes in the collagen structure, potentially weakening the overall tooth structure [18].

Light irradiation during bleaching has the advantage of accelerating the bleaching process, producing visible results faster, and demonstrating higher tooth bleaching efficacy. However, the heat generated by the light source can cause adverse effects, such as changes in the enamel surface and the tooth's microhardness [19]. Therefore, while light-based bleaching is effective, it can also pose potential risks to the dental hard tissues. This study aimed to verify the effectiveness and safety of cold plasma-based vital bleaching by evaluating the effects on the dentin hard tissue and structural stability by applying DW and cold plasma, which has a temperature similar to that of the human body, instead of conventional high-concentration oxidizing agents. Notably, this study was performed based on a 30-minute cold plasma exposure time, which has been reported to have a clinically significant whitening effect in previous studies [9-14, 21, 22].

Cold plasma can be used with various gases, including helium (He), Ar, oxygen (O_2), and nitrogen (N_2). The surface reaction and treatment characteristics vary depending on the gas used. In particular, it has been reported that gas-based cold plasma can activate molecules in DW, inducing an oxidation and bleaching reaction similar to H_2O_2. This suggests that DW can function as a practical bleaching agent when applied with plasma [20]. Furthermore, the tooth bleaching effect of cold plasma combined with a bleaching gel containing strawberry juice instead of a bleaching agent has been confirmed [21]. Cold atmospheric plasma generates short-lived reactive species, including hydroxyl radicals, that exert strong localized oxidative effects while rapidly dissipating, enabling effective penetration and interaction with dentinal tubules and subsurface structures without the need for conventional high-concentration oxidizing agents [20].

The study results confirmed the safety and applicability of cold plasma to vital bleaching and in preserving the dental hard tissues. After 30 minutes of sterile DW and cold plasma treatment, no statistically significant changes were observed in the surface microhardness of the dentin compared to the control group. Even after 30 minutes of cold plasma alone, no changes were observed. These suggest that cold plasma rapidly generates reactive species at low temperatures, inducing pigment oxidation and decomposition, thus preventing thermal damage [20]. This study suggests that cold plasma does not induce structural changes in the mineral components within the dentin matrix. Microhardness is related to the amount of mineral in the tooth, and a decrease in hardness can easily lead to tooth wear. While longer bleaching times can increase color change, microhardness is also decreased [22]. This study focused on assessing the structural stability of dentin, confirming no changes in surface microhardness or mineral composition. Although plasma generation generates a magnetic field, the results of this study are considered to be due to the influence of the low-cold atmospheric plasma itself. Because the cold plasma-based bleaching effect has already been demonstrated in previous studies, this study lacks color change analysis, a limitation. Nevertheless, the results showed no changes in mineral compositions or adverse effects on the dental hard tissues. This study confirmed that cold plasma is a stable energy source that can be applied to dental bleaching processes without causing thermal or chemical damage to dental hard tissues.

5. Conclusions

The effects of sterile DW and cold plasma on dentin hard tissues during the bleaching of denuded teeth were evaluated. After a 30-minute treatment, no significant changes were observed in the surface microhardness of the dentin or the tooth's mineral structure. Furthermore, cold plasma treatment alone did not exhibit any negative effects. This study also verified that structural stability is maintained after internal bleaching of denuded teeth. Considering these, cold plasma may be considered for non-invasive bleaching techniques that complement or replace existing oxidizing agents and/or light-based systems. This non-invasive bleaching technique not only improves color tone, but may be applied as an alternative or adjunctive bleaching technique that prioritizes the preservation of dental hard tissues while optimizing esthetics.

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