Tooth Enamel Evaluation After Tooth Bleaching With Hydrogen Peroxide Assisted by a DC Nonthermal Atmospheric-Pressure Plasma Jet

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***Abstract*—The purpose of this study is to evaluate the changes in dental enamel (morphology, elemental composition, microhardness, and roughness) after applying hydrogen peroxide in conjunction with a nonthermal plasma to bleach the teeth. Extracted human teeth were randomly placed in six groups. Two control groups (one group with no bleaching agent and no plasma treatment of the teeth and another one with only hydrogen peroxide as the bleaching agent) and four plasma groups (receiving hydrogen peroxide of varying concentrations 6%, 15%, 25%, and 35%, in conjunction with a plasma treatment) were prepared. The surface morphology before and after treatment was assessed using a scanning electron microscope (SEM), and the change in the elemental composition was analyzed by an energy-dispersive X-ray spectroscopy system. A total of 36 extracted teeth were used to evaluate the change in enamel microhardness and surface roughness. The use of hydrogen peroxide as a bleaching agent, even in the absence of plasma exposure, causes various etching patterns that are attributable to demineralization during the treatment process. These patterns are more pronounced as the hydrogen peroxide concentration increases. The surface roughness tests confirmed the findings from the SEM analysis. We only found minor essentially insignificant changes in the elemental composition of the enamel and in the surface microhardness as a result of the treatment using hydrogen peroxide and a cold plasma. The use of a cold plasma in conjunction with hydrogen peroxide of varying concentrations in tooth bleaching causes minor changes in the tooth enamel changes that are comparable to those resulting from the standard treatment using 35% hydrogen peroxide gel without a plasma.**

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***Index Terms*—Energy-dispersive X-ray spectroscopy (EDX), microhardness, nonthermal plasma, scanning electron microscope (SEM), surface roughness, tooth bleaching, tooth enamel.**

I. Introduction

TOOTH bleaching is one of the most commonly applied treatments in cosmetic dentistry [1]. Since the first bleaching of discolored pulpless teeth was described in 1864 [2], a variety of agents such as chloride, sodium hypochlorite, and hydrogen peroxide have been used, either alone or in combination with heat, laser radiation, or UV light [3]–[5]. In recent years, low-temperature ("cold") plasmas at atmospheric pressure in air (or in other gases and gas mixtures) have been shown to provide distinct advantages for tooth bleaching [6], particularly when used in conjunction with hydrogen peroxide [7]–[10]. This has been attributed in part to the generation of copious amounts of reactive radicals in the plasma, particularly of reactive oxygen species, such as $HOO^{\cdot}$, $^1O_2$, and $^{\cdot}OH$. It is believed that the presence of per-hydroxyl anions (HO_2^-) and $^{\cdot}OH$ are particularly critical in tooth bleaching [11], [12]. From a safety point of view, it is essential, however, to investigate the effect of tooth bleaching on the enamel. Enamel morphology, elemental composition [13], microhardness, and surface roughness are factors that characterize the enamel, and one needs to know to what extent the plasma-assisted tooth bleaching treatment may induce changes to the enamel [14]–[16]. The present study is devoted to a quantitative evaluation of such potential changes. Scanning electron microscopy (SEM) [17]–[19] and energy-dispersive X-ray spectroscopy (EDX) [20] were used to assess changes in the enamel morphology and elemental composition, and a microhardness [21] tester and a surface roughness tester were used to investigate changes in surface microhardness and roughness.

II. Materials and Methods

A. Plasma Device

The plasma device used in this work consists of two coaxial copper cylinders as electrodes, which are separated by a dielectric layer with a thickness of about 0.5 mm, as schematically shown in Fig. 1(a). The inner electrode is powered by a dc high-voltage power supply (negatively biased), whereas the outer electrode is grounded for safety considerations. The nozzle opening of the plasma device has a diameter of around 0.8 mm.

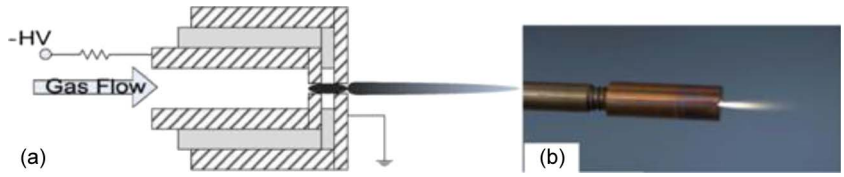

Fig. 1. (a) Schematic diagram. (b) Picture of a PMJ.

TABLE I
DETAILS OF THE TREATMENT GROUPS. GROUPS C–F RECEIVED 15-min PLASMA TREATMENT

Group	Treatment
A	No Treatment
B	35% H_2O_2 Alone
C	6% H_2O_2+Plasma
D	15% H_2O_2+Plasma
E	25% H_2O_2+Plasma
F	35% H_2O_2+Plasma

Further details of the plasma device and the electrical circuitry can be found in previous publications [10], [22], [23]. Compressed air at a flow rate of 5 slm is used as the working gas and is forced to flow through the inner electrode. The sustaining voltage of the plasma microjet (PMJ) is in the range of 400–600 V, with an operating current of 20–35 mA. Fig. 1(b) shows a picture of the PMJ working in air with a typical length of the plasma plume extending about 10 mm beyond the exit nozzle of the device [9]. The plasma can touch human skin with no adverse thermal, chemical, or electrical effect, and the entire device weighs less than 1 kg.

B. Tooth Bleaching Experiments

Dental gel (Beyond Technology Corporation, USA) with H_2O_2 at different concentrations as the bleaching agent was used in the experiments. Sixty human molars extracted for periodontitis reasons without caries and cracks were chosen. All teeth were cleaned and prepared by removing the roots, stored in a 0.1% thymol solution at 4 °C before been used. The teeth were randomly placed into six groups $(N = 10)$. The teeth in the blank control group (Group A) did not receive any treatment, whereas the teeth in the negative control group (Group B) received 35% H_2O_2 dental gel, but no plasma treatment. The teeth in the other four groups (groups C–F) received dental gel with various H_2O_2 concentrations (6%, 15%, 25%, and 35%) and were also subjected to a standardized nonthermal plasma treatment for 15 min (see Table I). The dental gel applied to the teeth in groups B–F was a 1-mm-thick layer and was replenished every 30 s in groups C–F to ensure adequate coverage of each tooth during the 15-min plasma treatment.

C. SEM and EDX

Three teeth were chosen, and each tooth was longitudinally cut into six bar-shaped test specimens with a cross-sectional dimension of 2 mm × 3 mm. They were divided into six groups as above. The surfaces of the specimen were cleaned and dried at 37 °C overnight. In an effort to achieve better electrical conduction of the samples, the sample surface was sputter coated with a 20-nm gold layer using a low deposition rate, cooling of the substrate, and a sufficient distance between the target and the sample to avoid sample damage [17]. The surface morphology and the elemental composition were determined before and after different treatments. The differences in the atomic abundance of O, Ca, and P between the untreated and treated samples were statistically analyzed with the analysis of variance (ANOVA) method via SPSS (Statistical Package for the Social Sciences. IBM Corp., V14). The changes were considered significant when the value of P was less than 0.05.

D. Surface Roughness and Microhardness

The extracted human teeth were cut longitudinally into bar-shape test specimens and embedded in epoxy resins with an enamel cross-sectional surface area of 3 mm × 3 mm. In order to keep the baselines of microhardness and surface roughness measurements at the same level, the enamel was polished before the plasma treatments. The enamel surfaces were first manually polished in sequence with 800#, 1000#, and 1200# water-proof abrasive paper and finally polished with a polishing machine. A total of 36 specimens were used to evaluate the change of enamel microhardness and surface roughness. Microhardness and roughness measurements of the enamel were performed before and after the plasma or pure hydrogen peroxide treatment. The microhardness was measured three times for each specimen using a Shimadzu HMV tester with a Knoop indenter at a load of 0.9807 N for 15 s [9]. The surface roughness was measured three times for each specimen using a Mitutoyo SJ-400 portable digital roughness tester with three steps, 0.08 mm per step. The mean values of the change in microhardness and the change in surface roughness from before to after the treatments were evaluated for statistical significant differences via ANOVA, and changes were considered significant if P was less than 0.05.

III. RESULTS AND DISCUSSION

A. SEM Analysis

Fig. 2 shows the enamel surface morphology of a representative tooth from each group at a magnification of 3000. The morphology of any enamel surface covered with the bleaching agent [see Fig. 2(b)–(f)] shows a variety of etching patterns not found for the teeth in the blank control group [see Fig. 2(a)]. These changes are attributed to demineralization during the treatment process. All treated teeth show signs of erosion, such as the appearance of "doughnut" structures, enhanced porosity, and hairpin-shaped lines [24]. Whereas the teeth in the plasma-treated groups with a comparatively low concentration of hydrogen peroxide [see Fig. 2(c) and (d)] show etching

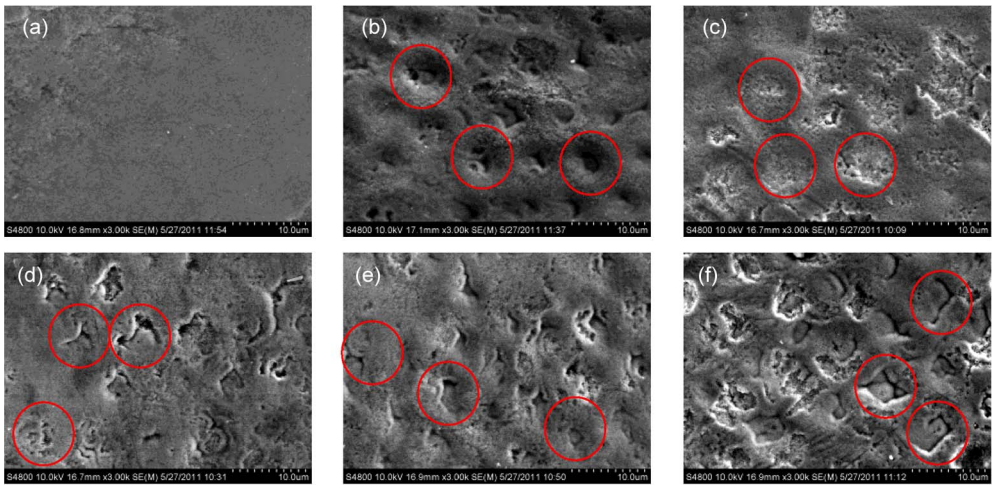

Fig. 2. SEM micrograph of enamel surfaces after treatment (magnification of 3000): (a) no treatment; (b) 35% H_2O_2, no plasma; and (c)–(f) plasma treatment with H_2O_2 at concentrations of 6%, 15%, 25%, and 35%, respectively. (Red circles mark the typical enamel demineralization morphology: "doughnut" structures, enhanced porosity, and hairpin shaped.)

patterns and a porosity similar to the teeth in the negative control group [see Fig. 2(b)], we find a higher degree of porosity and a higher density of etched structures in teeth that were plasma treated in the presence of higher H_2O_2 concentrations [see Fig. 2(e) and (f)].

The demineralization (erosion) patterns are related to the distribution of various enamel crystals (e.g., carbonated apatite, hydroxyapatite, and fluorapatite) in the enamel rods (enamel prisms). The actual arrangement of the crystals in each enamel rod is rather complex. However, near the head of the enamel rod, the crystals are arranged parallel to the long axis of the rods. The central regions of the enamel rods are richer in carbonated apatite, which is more susceptible to acid demineralization than other crystallites found in enamel rods. Therefore, demineralization preferentially occurs in the central regions at the head of the enamel rods and then progresses along the central core, showing a doughnut-shaped (for enamel rods with straight crystal sections) or a hairpin-shaped (for enamel rods with tilted crystal sections) erosion pattern. Higher degrees of erosion are observed for higher concentrations of hydrogen peroxide assisted by the plasma treatment. However, the highest morphological change due to erosion observed in this study [35% H_2O_2 and plasma treatment, see Fig. 2(f)] is similar to that required in dental bonding procedures [18]. Therefore, enamel surface morphological changes due to the plasma and H_2O_2 treatment are considered acceptable, and the degree of change can be controlled by the concentration of H_2O_2 used in the process.

B. EDX Evaluation

The EDX data in terms of the atomic abundance of Ca, O, and P (primary elemental compounds of tooth enamel) are displayed in Fig. 3 for teeth from all six groups. The changes induced by the treatment are generally minimal, except perhaps for the plasma treatment with 35% H_2O_2, which shows an enhanced O concentration and a decline in the P and Ca concentrations. The largely unchanged Ca concentration suggests that the resistance to acid dissolution is acceptable

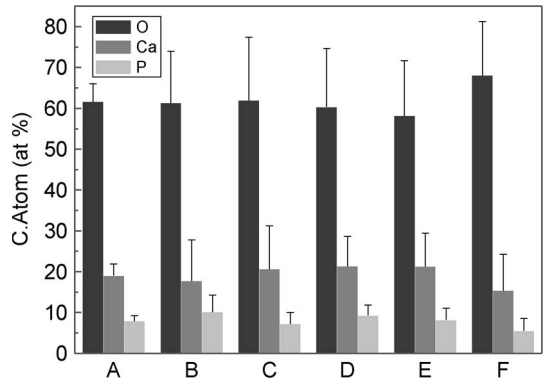

Fig. 3. Mean value and the standard deviations of O/Ca/P atomic abundance on the enamel surface for three teeth in each of the six groups after treatment. The elemental composition shown for the blank control group represents that of teeth in all groups prior to any treatment. O/Ca/P atomic abundance between the blank control group and each treated group showed no significant difference.

under these experimental conditions. No significant difference in the O/Ca/P atomic concentrations between the untreated and treated samples was observed. It is worth noting that, to the best of the authors' knowledge, there were no prior reports in the literature that studied the concentration of several elements on the enamel surface after exposure to a nonthermal plasma.

C. Microhardness

The microhardness of the enamel surface was reduced after a 15-min treatment. However, the change was barely outside the combined uncertainties of the measured values before and after the treatment (see Table II and Fig. 4). Moreover, no significant difference was found in the change in microhardness between the teeth in the blank control group and those in the treated groups ($p > 0.05$). The reduction in the microhardness of the teeth in the blank control group may be attributed to the fact that the teeth were stored in liquid and dried before each microhardness test. Thus, we conclude that the change in the microhardness was not significantly affected by the use of 35% hydrogen peroxide alone or by the use of H_2O_2 at different concentrations in conjunction with a plasma treatment.

TABLE II
MEAN VALUES AND STANDARD DEVIATIONS OF ENAMEL MICROHARDNESS OF SAMPLES IN VARIOUS GROUPS BEFORE AND AFTER THE TREATMENTS OF 15 min. MEAN VALUES OF THE CHANGES IN MICROHARDNESS (AND STANDARD DEVIATIONS) WITHIN EACH GROUP ARE ALSO INCLUDED AS A SEPARATE COLUMN

Group	Initial	Final	Change
A	358.87±15.23	319.67±22.17	39.20±19.88
B	302.83±55.65	250.22±63.35	44.20±26.96
C	335.78±23.30	261.56±30.72	64.20±16.89
D	312.00±28.61	267.22±35.76	39.40±12.90
E	336.67±31.66	283.22±9.35	41.46±20.84
F	314.29±51.16	287.29±55.25	46.40±20.47

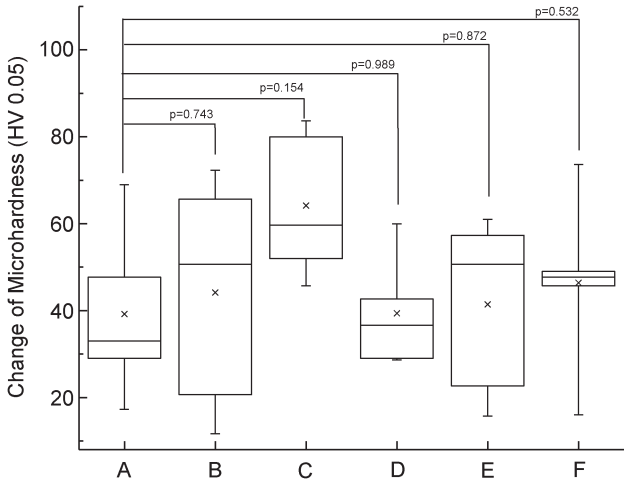

Fig. 4. Box and whisker plot of changes in microhardness for the treated and control groups. Upper, median, and lower ends of each box represent the 75th, 50th, and 25th percentile, respectively. Whiskers extend to smallest and largest data points. Crosses (×) are used to represent the mean values in each group.

TABLE III
MEAN VALUES AND STANDARD DEVIATIONS OF ENAMEL SURFACE ROUGHNESS OF SAMPLES IN VARIOUS GROUPS BEFORE AND AFTER THE TREATMENT OF 15 min. MEAN VALUES OF THE CHANGES IN SURFACE ROUGHNESS (AND STANDARD DEVIATIONS) WITHIN EACH GROUP ARE ALSO INCLUDED AS A SEPARATE COLUMN

Group	Initial	Final	Change
A	0.062±0.031	0.224±0.055	0.162±0.084
B	0.037±0.018	0.208±0.055	0.158±0.063
C	0.080±0.063	0.225±0.076	0.148±0.078
D	0.068±0.037	0.200±0.072	0.128±0.059
E	0.047±0.014	0.157±0.043	0.106±0.050
F	0.061±0.053	0.176±0.066	0.102±0.031

D. Surface Roughness

The change in surface roughness inferred from the SEM analysis (see Fig. 2) was confirmed by the data obtained from the surface roughness tester (see Table III and Fig. 5). The tooth surface roughness increased after a 15-min plasma exposure. Slight differences that were not statistically significant were found in the change in surface roughness among the teeth in the blank control group, the teeth in the negative control group, and the teeth in the various plasma groups.

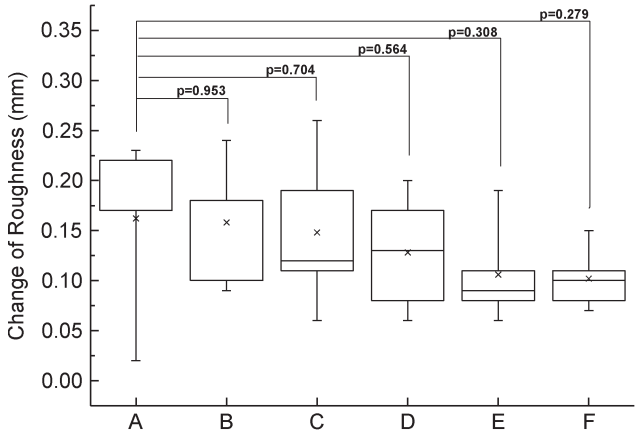

Fig. 5. Box and whisker plot of changes in roughness for the treated and control groups. Upper, median, and lower ends of each box represent the 75th, 50th, and 25th percentile, respectively. Whiskers extend to smallest and largest data points. Crosses (×) are used to represent the mean values in each group.

IV. CONCLUSION

Various concentrations (6%–35%) of hydrogen peroxide were used in a tooth bleaching treatment in conjunction with a dc nonthermal atmospheric-pressure plasma jet. Changes in enamel morphology, elemental composition, microhardness, and surface roughness were analyzed before and after the treatment. Results indicate that the use of hydrogen peroxide as a bleaching agent, even in the absence of plasma exposure, resulted in the appearance of various etching patterns that were attributed to demineralization during the treatment process. The etching patterns are more pronounced as the hydrogen peroxide concentration increases. We observed some minor changes in the elemental composition of the enamel and in the surface microhardness, which are deemed not significant.

Overall, we conclude that the use of a plasma in conjunction with hydrogen peroxide of varying concentrations causes enamel changes that are quite comparable to those resulting from the standard treatment using 35% hydrogen peroxide gel, which are considered acceptable.

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