Changes in Bovine Enamel after Treatment with a 30% Hydrogen Peroxide Bleaching Agent

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The purpose of this study was to investigate the effect of long-period exposure of 30% hydrogen peroxide on bovine enamel. Polished labial surfaces were scanned using an atomic force microscope after bleaching for 120 hours. Compositional change and microhardness of the enamels were evaluated using a Fourier transform Raman spectrophotometer and Vickers hardness tester. The same tests were performed on enamels stored in distilled water. In the FT-Raman spectra of both the un-bleached and bleached enamels, peaks remained unchanged except for negligible decrease in intensity. As for microhardness, it significantly decreased after bleaching when compared to the original value (p<0.0001). However, the microhardness values of enamels stored in the bleaching agent and distilled water did not show any statistical difference. Based on the results of this study, the use of 30% hydrogen peroxide solution for dental bleaching should be safe due to its negligible effects on tooth morphology and structure.

INTRODUCTION

The use of bleaching agents to whiten teeth has a long history. Hydrogen peroxide is one such bleaching agent, of which its efficacy in whitening discolored teeth was established by the early 1900s³. The reason for dental discoloration is generally due to complex chemical and physical interactions of teeth with stain-causing materials such as coffee and soft drinks². Smoking is also a very common cause of discoloration. Hydrogen peroxide is an oxygen-rich solution that readily decomposes when it encounters materials with which it can react. Decomposed hydrogen peroxide releases oxygen-free radicals that initiate complicated oxidation processes. In the course of the oxidation process, dark-colored organic molecules change to light-colored ones. However, the exact mechanism of hydrogen peroxide decomposition has yet to be determined³-⁵.

The effects of bleaching agents on teeth, in terms of color change and surface modification, have been evaluated by many researchers⁶-¹⁰. Researchers generally agree that bleaching agents effectively reduce or eliminate dental discoloration. However, reported conclusions concerning surface modification vary and differ¹¹-¹⁴. In many cases, the results were affected by the resolution and magnification of the scanning electron microscope. Unlike a scanning electron microscope, an atomic force microscope (AFM) has many favorable features such as less need for perfect dehydration and surface coating with conductive materials, the ability to operate under all sample conditions, and availability of 3D images. Atomic-level resolution and nanometer-order observations without surface damage during sample scanning make AFM an attractive alternative for extremely high-resolution images¹⁵-¹⁷.

As an alternative technique to conventional dispersive Raman spectroscopy, Fourier transform Raman (FT-Raman) spectroscopy is increasingly recognized as a significant analytical method for biomedical applications⁸,¹⁰. FT-Raman spectroscopy is a technique that utilizes Raman signals. The signals contain a significant amount of important information regarding the composition and structure of materials at molecular level. Any changes in a tooth will affect the states of molecules and vibrational modes; these changes will in turn be reflected in the Raman signals. One of the main advantages of using Nd: YAG laser as a light source is the elimination of fluorescence, which occurs due to the electronic absorption of the excitation radiation, even though this is a controversial topic still hotly debated²⁰-²².

The softening of tooth surface after treatment with bleaching agent has been investigated by many researchers and has made dentists conscious of the safety of using bleaching agents on their patients’ teeth³,¹⁰. Mineral loss from tooth surface, which alters the morphology of the enamel, reduces microhardness – depending on the content of mineral loss⁴,²⁰. Mineral loss has a close relation to our
daily life. Consumption of soft drinks and fruit juices for just a few minutes readily dissolves minerals from the teeth in amounts that are similar to the mineral loss accompanying the immersion of teeth in a bleaching agent for several hours or longer
draft.

The purpose of this study was to investigate the effect of a long-period exposure of a bleaching agent, 30% hydrogen peroxide, on dental enamel. Surface modifications, compositional changes, and softening of the teeth in the course of the bleaching process were evaluated.

MATERIALS AND METHODS
Five non-carious bovine incisors were finely polished using SiC paper and then ultra-finely polished using diamond paste. Before treatment with a bleaching agent and for reference purpose, the labial side of the enamels - at 500×500 nm dimension - was scanned using the tapping mode of an atomic force microscope (Nanoscope Multimode, Digital Instruments, Santa Barbara, USA). The tapping mode causes no surface damages and produces extremely fine images. The scanned teeth were sectioned; half of each tooth was used for bleaching and the other half stored in distilled water as control. The probe for the tapping mode was composed of a cantilever and cone-shaped tip made with etched silicon. The spring constant of the cantilever was 0.02-0.1 N/m. Half of the sectioned specimens were bleached using a bleaching agent (Sigma Chemical Co., St. Louis, USA), 30% hydrogen peroxide, for 120 hours without interruption. After bleaching for 120 hours, the specimens were removed from the bleaching agent, rinsed with tap water, and dried in air. The surface morphology of the bleached specimens was then re-evaluated. The rest of the sectioned specimens, which were stored in distilled water for 120 hours, were tested in the same way for comparison.

Fresh and non-carious bovine incisors were finely polished using SiC paper, cut properly to fit into the sample holder of a spectrophotometer, sonicated for five minutes three times, and then attached to a sample holder. FT-Raman spectra of the unbleached enamels were recorded using a FT-Raman spectrophotometer (IFS120HR/FRA106, BRUKER, Germany) equipped with a diode-pumped Nd:YAG laser as light source. The spectra were obtained using 100 scans at laser power less than 50 mW. After recording the FT-Raman spectra, the samples were removed from the holder and immersed in 30% hydrogen peroxide solution for 120 hours at room temperature without interruption. The samples were then removed from the solution, rinsed with tap water several times without brushing, and then dried in air. The dried specimens were attached to a sample holder and FT-Raman spectra were recorded again using the same equipment and conditions mentioned above.

To measure the microhardness of unbleached and bleached enamels, seven non-carious incisors were selected, finely polished using SiC paper and then sonicated several times. Using a Vickers hardness tester (FM-7, FUTURE-TEC Inc., Japan), seven indentations were made on the surface along one straight line. Each indentation was parted by 0.5 mm. To make the indentation, a 15-second dwelling time and a 200-g load were chosen. The measured microhardness values of the unbleached enamel were used as the reference value. Next, teeth were sectioned and half of the sectioned teeth were immersed in 30% hydrogen peroxide solution for 24 hours without interruption. The immersed enamels were then removed and washed with tap water. Excess water was removed with a cotton swab and the enamels dried in air. The same microhardness measurement was performed under the same conditions as previously mentioned. This time, the measurements were performed 0.5 mm apart from the previously measured spot. After this set of measurements, the teeth were immersed immediately in the bleaching agent again for 48 hours. The same processes mentioned above were repeated until the total bleaching time reached 120 hours. For comparison, the rest of the sectioned specimens were stored in distilled water and their microhardness measured in the same way as above. The acquired data for microhardness were analyzed by one-way ANOVA followed by Duncan’s multiple range test for variable at 0.05 level of significance. A t-test was performed to determine the statistical difference between the specimens stored in distilled water and the bleaching agent.

RESULTS
Figs. 1 and 2 show, respectively, the surfaces of the enamels after 120 hours of storage in distilled water and the bleaching agent. The scanned dimension on each surface was 500×500 nm. The edges of the exposed enamel crystals were rounded, periodically un-

Fig. 1 AFM image of enamel immersed in distilled water for 120 hours in 500×500 nm dimension.
Fig. 2 AFM image of enamel bleached using 30% hydrogen peroxide for 120 hours in 500×500 nm dimension.

Fig. 3 FT-Raman spectra of unbleached and bleached 120-hour enamels. Peaks are associated with phosphate and carbonate ions in hydroxyapatite.

Fig. 4 FT-Raman spectra of unbleached and bleached 120-hour enamels. Peaks are associated with organic phase (C=H stretching mode).

Fig. 5 Change of microhardness of teeth stored in different media and periods of time.

**DISCUSSION**

When hydrogen peroxide interacts with a tooth, it decomposes into hydroxyl radicals or into water and oxygen molecules, depending on the mechanism of hydrogen peroxide decomposition\(^3,10\). The free
radicals released are unstable and immediately seek an available target with which they may react. The reaction may decompose organic materials, including dental stains on enamel, from larger, longer-chained, darker-colored molecules into smaller, shorter-chained, lighter-colored molecules. In the course of decomposition, a color change occurs on the enamel surface and the decomposed organic materials are dissolved in the hydrogen peroxide solution\textsuperscript{8,9,26}. Since organic materials are distributed mainly in the inter-zones of inorganic structures, the removal of organic materials makes the surface uneven. The organic materials can be proteins, lipids, or dental staining substances. Hydrogen peroxide can also interact with inorganic materials. It then dissolves the enamel surface gradually by removing the mineral elements. At the same time, hydrogen peroxide penetrates the subsurface of enamel along the enamel’s intra- or inter-prismatic regions. Organic materials in these regions will act as channels for hydrogen peroxide to penetrate through. As reported in other studies, the penetration depth of the bleaching agent will increase due to increased enamel permeability as the storage period in hydrogen peroxide increases\textsuperscript{27,28}. Based on this interaction, grooves on the enamel surface are deepened and the ends of the enamel crystals gradually change from rounded to conical.

As observed in the AFM images, the modifications on the enamel surfaces were easily identified because AFM is capable of generating three-dimensional images with atomic-level resolution as well as being capable of detecting surface alterations in nanometer order. On the other hand, in SEM, the findings of microscopic alterations on enamel mostly depend on the resolution and magnification of the equipment. However, in many cases, the magnifications employed in the photographs were not high enough to show the microscopic alterations. Thus far, numerous studies have evaluated the effect of peroxide-containing bleaching agents on tooth morphology. On the whole, the reported results were inconsistent. Some studies have found insignificant modifications on the enamel surface but other investigators have described otherwise\textsuperscript{11-14}. Since we could barely observe any morphological modification in nanometer order in this study, surface modifications due to the bleaching agent should be minimal.

One important parameter of FT-Raman spectroscopy on biological tissues is the laser power. High levels of laser power on sample surfaces are destructive to hydrated tissues and may affect Raman signals due to temperature increase during measurement. Low levels of laser power may reduce damage but may induce structural changes arising from dehydration due to extended data acquisition time. The Raman intensity is also affected by crystal orientation\textsuperscript{29}. Variations in orientation angle of enamel crystals can change Raman intensity and profile. Positioning the sample at the same place and direction toward the incident laser beam throughout the experiment is important to prevent misinterpretation of the acquired data. From the acquired FT-Raman spectra in this study, treatment with 30% hydrogen peroxide solution for 120 hours did not induce any significant compositional changes except for a minor loss of organic and inorganic materials. No peak shifts nor appearance or disappearance of Raman peaks occurred, which implies there were no changes in tooth composition.

Treatment with 30% hydrogen peroxide solution also caused a slight softening of the treated enamel surfaces. The decrease in microhardness could be attributed to surface degradation, resulting from the complicated oxidative process of free radicals. In the course of surface degradation, the overall mineral content was reduced. However, the volume of mineral loss from the tooth was not considered significant\textsuperscript{30}. Since microhardness is directly related to the mineral content of enamel, mineral loss did reduce the microhardness of enamel\textsuperscript{11,14}. In this study, microhardness reduction during the bleaching process was maximal in the first 24 hours. After 24 hours, even though the surface continued to soften gradually, the reduction rate decreased as the hours in the bleaching agent increased. Since the specimens stored in two different storage media did not show significant statistical differences between them up to 120 hours (a very long treatment period from clinical perspective), softening of the surface due to bleaching may not be clinically significant.

From the study, it is clear that hydrogen peroxide certainly dissolves organic materials and minerals from teeth and makes the surface of enamel soft and less compact. However, this is not sufficiently conclusive to therefore assert that this bleaching agent is unsafe for teeth because the amount of mineral loss after 120 hours’ bleaching was as little as the amount of mineral loss caused by 2-2.5 minutes’ consumption of soft drink or fruit juice\textsuperscript{14,25}. Instead, further study is needed to determine if the bleaching-induced modification on enamel surface triggers any unexpected effect on teeth.

REFERENCES
4) Albers H. Lightening natural teeth. ADEPT Report


