

Effect of 10% Carbamide Peroxide on Dentin

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국문초록

최근 들어 심미적 목적으로 치아 미백제를 이용한 치아 미백술이 널리 사용되고 있다. 현재까지 치아 미백제의 정확한 기전은 밝혀져 있지 않지만 미백제로부터 여러 가지 방법으로 분해된 free radical의 복잡한 산화작용에 의한 것으로, 치아변색의 원인물질의 화학적 구조가 바뀌어짐으로써 희게 되는 것으로 이해하고 있다. 본 연구의 목적은 치아 미백제에 의한 상아질의 특성변화를 관찰하고 미백제의 안전성을 평가하고자 하였다. 이를 위해 10% carbamide peroxide를 1일에 6시간 2주동안 사람의 소구치 법랑질에 적용한 후 상아질에서 나타나는 형상변화, 경도변화, 조성변화 및 무기질 함량변화를 주사전자현미경(SEM), 미세경도기, FT-Raman 분광기 그리고 전자탐침미세분석기(EPMA)를 이용하여 분석하고 다음과 같은 결과를 얻었다.

법랑질 표면에 도포한 10% carbamide peroxide가 상아질로 침투하여 미친 영향은 증류수에만 담가둔 시료와 비교하였을 때 형상변화의 측면에서는 차이를 발견할 수 없었다. Peritubular dentin과 intertubular dentin의 표면에서는 nanometer range에서 아무런 변화를 발견할 수 없었다. 미백처리한 치아와 증류수에만 담가둔 치아의 상아질에서 통계적으로 유의한 정도의 차이를 발견할 수 없었다. FT-Raman 분석결과 상아질 구성성분 중 어떤 변화도 분광학적으로 관찰되지 않았다. 유기질에 의한 peak와 무기질에 의한 peak 모두 무시할 수 있을 정도의 Raman intensity 변화만 관찰되었다. 무기질 함량변화를 측정된 결과 아무런 처리도 하지 않은 치아의 상아질, 증류수에만 담가둔 치아의 상아질 그리고 미백후 증류수에 담가둔 치아의 상아질에서 무기질 총량이 각각 98.73 ± 1.89 , 98.56 ± 2.11 , 97.47 ± 2.51 로 나타났다. 이들 값은 통계학적으로 유의한 차이를 보이지 않았다.

이상의 결과들로 볼 때 10% carbamide peroxide 미백이 상아질에 미치는 영향은 극히 미약하다고 판단되어 상아질에는 안전한 것으로 생각된다.

주요어 : 치아 미백, 10% carbamide peroxide, 상아질, FT-Raman 분광기, 미세경도

I. INTRODUCTION

At-home bleaching of a vital teeth with 10% carbamide peroxide has become a routine dental procedure due to a simple and noninvasive treatment for discolored teeth. The reason for dental discoloration is generally originated from complex chemical and physical interactions of teeth with various stain-causing materials and aging. Intrinsic teeth stains are developed from defects in tooth development, brown fluorosis and use of tetracycline. Extrinsic stains are localized mainly in the pellicle and originate from chromogenic foods, drinks and smoking^{1,2}. Carbamide

peroxide is a bleaching agent, which contains hydrogen peroxide as an active whitening agent. When hydrogen peroxide reacts with discolored teeth, it easily decomposes and releases oxygen free radicals. The released oxygen free radicals initiate the oxidation process in the teeth. However, the exact mechanism of decomposition is not fully known^{3,4}.

The effects of 10% carbamide peroxide on dentin have not been explored as much in the case of enamel. At-home bleaching has been reported to change the color of dentin for different situations in which dentin was exposed to the bleaching agent directly or indirectly⁵⁻⁷. Surface morphology changes of dentin

were not observed from scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM)⁷⁻⁹. Microhardness values of dentin were reported to decrease after bleaching. However, microhardness values for sound and demineralized dentin submitted to the bleaching agent did not show statistical differences¹⁰⁻¹². A significant reduction in the Ca/P ratio was found after the direct treatment of 10% carbamide peroxide to dentin¹³. Also, carbamide peroxide was reported to penetrate the pulp chamber through the coronal wall, and the concentration of an active hydrogen peroxide was toxic to cultured cells¹⁴⁻¹⁶.

In spite of general use, studies concerning the effects of carbamide peroxide bleaching on dentin are still limited. The purpose of this study was to test the safety of 10% carbamide peroxide solution on dentin by examining the effect on the surface morphology, microhardness, chemical structure and content of mineral elements of human dentin.

II. MATERIALS AND METHODS

Fresh and non-carious human premolars (n=25), extracted for orthodontic reasons, were used in this study. Teeth were stored in 0.1% thymol solution at 4 after extraction. Teeth were cut in half longitudinally from buccal, and roots were removed. Teeth were cleaned and sonicated with distilled water for one minute several times. The cut surfaces were treated with nail varnish to prevent reaction with bleaching agent and water. Half of each group of sectioned teeth were stored in distilled water for 2 weeks to serve as control. The rest were bleached using 10% carbamide peroxide gel (Ultradent Products, South Jordan, USA). The tooth surface was spread with a bleaching gel for 6 hours/day for 2 weeks. After bleaching for 6 hours, teeth were washed in running water and stored in distilled water until the next bleaching. After the completion of bleaching and storing in distilled water, teeth were cleaned with running water without brushing. In a cylindrical container, orthodontic resin was filled. Each sectioned tooth was submerged into the resin by making the cut surface the top and pressed the tooth enough to be fully submerged into the resin. The size of the resin container was 10 mm in width and 5 mm in depth. The surface was slightly polished using SiC papers and then ultra-finely polished using diamond

pastels to remove the coated nail varnish. The polished teeth were cleaned ultrasonically in distilled water for 5 minutes.

Scanning Electron Microscope (SEM)

The cleaned teeth were dried in a vacuum dryer for 2 days. Dried teeth were gold-coated and their surface was observed using a SEM (S4200, Hitachi, Japan) at various magnifications.

Microhardness

Whole teeth (n=10) were selected, sectioned and polished as described above before imbedding into the orthodontic resin. The microhardness was measured before bleaching or storing in distilled water. Using a Vickers hardness tester (FM-7, FUTURE-TEC Inc., Japan), three indentations were made on the predetermined surface of each specimen. To make the indentations, a 15-second dwell time and 200-g load conditions were chosen. Following the measurements, the cut surfaces, at which the microhardness measurement was performed, were treated with nail varnish. Teeth were divided into two groups: one group (n=10) was bleached and the other group (n=10) was stored only in distilled water for 2 weeks. After completion of bleaching and storing in distilled water, teeth were submerged into the resin, polished and cleaned as described above. Three indentations were made on the pre-measured site of each specimen with the same dwell time and load conditions as before. Difference in the microhardness among dentins was analyzed using one-way ANOVA at a p value of 0.05.

FT-Raman spectrophotometer

To evaluate any compositional change in dentin after bleaching, teeth were randomly selected from the specimens used for the microhardness measurements. The surface of each tooth was cleaned with distilled water several times and dried in air. FT-Raman spectra of the unbleached (stored only in distilled water) and bleached dentins were recorded using a FT-Raman spectrophotometer (IFS120HR/FRA106, BRUKER, Germany) equipped with a diode-pumped Nd:YAG laser as a light source. The

spectra were obtained using less than 50 mW laser power and 100 scans. The scanned wavenumber ranged from 50 to 3500 cm^{-1} . The recorded Raman intensity was normalized for comparison.

Electron Probe Microanalyzer (EPMA)

Seven teeth were randomly selected and sectioned longitudinally. Half of them were bleached as described above, and the rest were stored in distilled water for 2 weeks. Teeth were then cleaned after completion of bleaching and storing in distilled water and put in a vacuum dryer for 2 days. Another 5 specimens that were not bleached or stored in distilled water were selected for reference. Two random points on the dentin of each specimen were selected and subjected to a point analysis using an electron probe microanalyzer (EPMA) equipped with a wave-

length dispersive spectrometer (WDS) (EPMA 1600, Shimadzu, Japan). The quantity of calcium, phosphorus, fluoride, magnesium and zinc were determined. The examination beam size was 1 μm with a 20 kV acceleration voltage. Hydroxyapatite and fluorapatite were used as the standards for quantitative analysis. The actual weight percentage of the elements was calculated from the original EPMA values, which were given as oxides (CaO, P_2O_5 , MgO and ZnO). The acquired data were analyzed by one-way ANOVA followed by Tukeys studentized range test for variable at a p value of 0.05.

III. RESULTS

Figs. 1 and 2 show the sectioned surface of the peritubular dentin and the wall of the intertubular dentin after storing in distilled water. Figs. 3 and 4

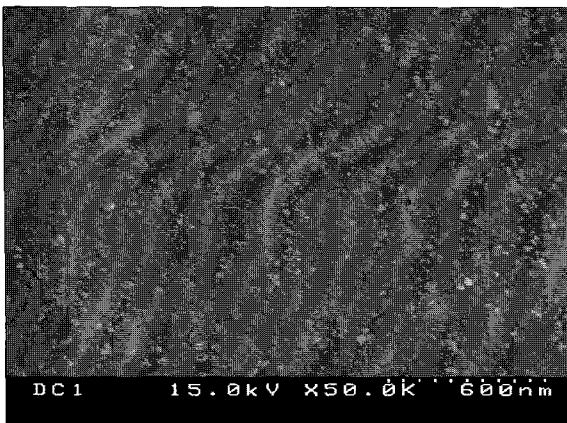


Fig. 1. The sectioned surface of the peritubular dentin after storing in distilled water.

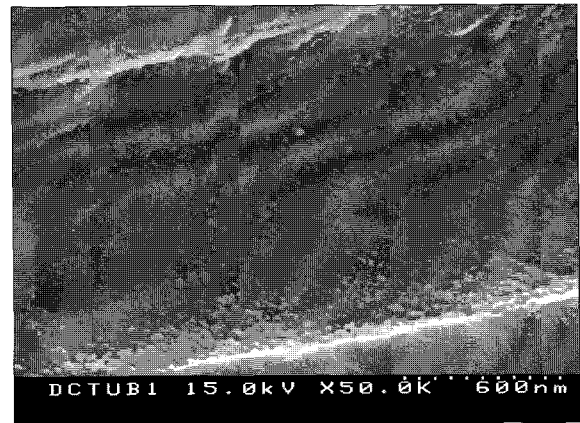


Fig. 2. The wall of the intertubular dentin stored in distilled water after bleaching.

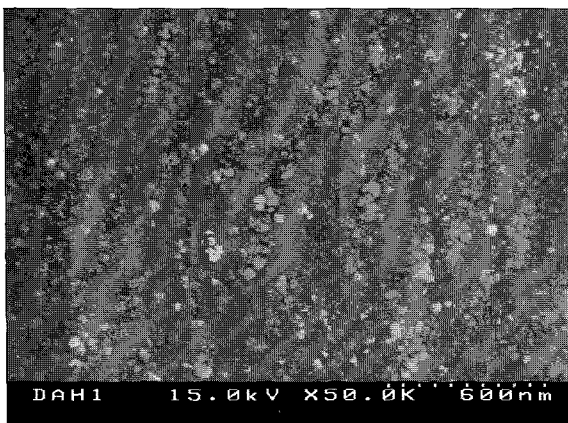


Fig. 3. The sectioned surface of the peritubular dentin after storing in distilled water.



Fig. 4. The wall of the intertubular dentin stored in distilled water after bleaching.

Table 1. Surface microhardness (HV) of dentins stored in distilled water and stored in distilled water after bleaching

Control	Group 1	Group 2
62.53±5.39*	61.96±5.87*	62.18±6.47*

Control : No water, no bleaching.

Group 1 : Soaked in distilled water for 2 weeks.

Group 2 : Bleached for 6 hours/day for 2 weeks. After bleaching for 6 hours, teeth were soaked in distilled until the next bleaching.

* No significant difference (one-way ANOVA, $p < 0.05$).

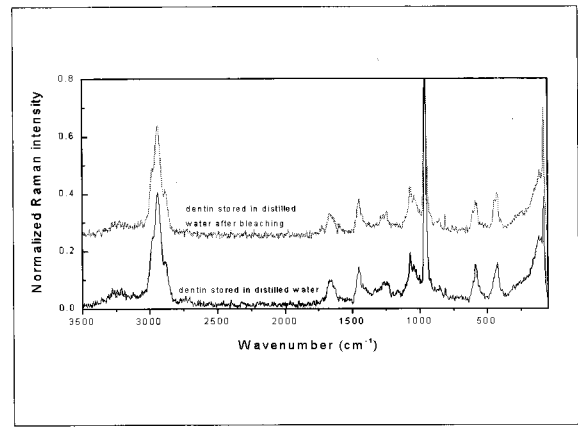


Fig. 5. Normalized Raman spectra of dentin stored in distilled water and stored in distilled water after bleaching with 10% carbamide peroxide.

Table 2. The means and standard deviations of the mineral elements of dentins for different treatment of teeth

Element	Control	Group 1	Group 2
Ca	38.53±1.73	38.42±1.45	38.26±1.83
P	18.64±0.73	18.49±0.77	19.01±0.67
Mg	0.71±0.08	0.67±0.06	0.78±0.10
F	0.20±0.10	0.19±0.10	0.22±0.07
Zn	0.03±0.01	0.06±0.05	0.02±0.01
Total*	98.73±1.89	98.56±2.11	97.47±2.51

Control : No water, no bleaching.

Group1 : Soaked in distilled water for 2 weeks.

Group2 : Bleached for 6 hours/day for 2 weeks. After bleaching for 6 hours, teeth were soaked in distilled until the next bleaching.

* No Significant difference (one-way ANOVA, $p < 0.05$).

show the sectioned surface of the peritubular dentin and the wall of the intertubular dentin stored in distilled water after beaching. Dentin crystals were compact, and the grain boundaries in Figs. 1 and 3 were not clear. The wall of the intertubular dentins in Figs. 2 and 4 remained intact and did not show any recognizable change whether teeth were bleached or not. Table 1 shows the surface microhardness (HV) of dentins. Compared to the original (no water, no bleaching) microhardness, values of dentins stored only in distilled water (case 2) and stored in distilled water after bleaching (case 3) were almost identical. The microhardness among cases did not show any statistically significant difference (one-way ANOVA, $p < 0.05$). Fig. 5 shows

the FT-Raman spectra of dentins stored only in distilled water and stored in distilled water after bleaching. Numerous peaks were associated with both the organic and inorganic phases of dentin. The peaks 430 cm^{-1} (PO_4^{3-} bending mode), 590 cm^{-1} (PO_4^{3-} bending mode) and 960 cm^{-1} (PO_4^{3-} stretching mode) were associated with the inorganic phase. Similarly, the peaks 1043 cm^{-1} (CO_3^{2-} bending mode) and 1068 cm^{-1} (CO_3^{2-} stretching mode) were presented due to the carbonate group. The peaks $1240\sim 1270\text{ cm}^{-1}$ (Amide III band), 1450 cm^{-1} (C=H bending mode), $1640\sim 1670\text{ cm}^{-1}$ (Amide I band) and 2950 cm^{-1} (C=H stretching mode) were due to the organic phase. The peaks 1240 , 1450 and 1660 cm^{-1} were likely due to the amide bands of collagen.

The bleached dentin did not exhibit any spectral change compared to the dentin stored only in distilled water except for a minor change in Raman intensity. The spectra were well defined and easily identified. Table 2 shows the means and standard deviations of the mineral elements of dentin for different teeth conditions based on the EPMA data. The total content of mineral elements were 98.73 ± 1.89 , 98.56 ± 2.11 and 97.47 ± 2.51 . The difference of the total mineral contents among cases did not show statistical significance.

IV. DISCUSSION

When hydrogen peroxide encounters other materials, it readily decomposes and releases oxygen free radicals. The released oxygen free radicals readily interact with tooth in the surface and penetrate into the subsurface. Hydrogen peroxide, an active compound of carbamide peroxide, is known to penetrate into the pulp chamber through the coronal wall of the tooth¹⁴⁻¹⁶. The degree of alterations in the tooth depends on the length of treatment and the concentration of the bleaching agent^{17,18}. In an SEM study evaluating the effect of 10% carbamide peroxide gel on enamel and dentin, no surface morphological changes were observed after 72 hours of contact with the bleaching agent⁹. In our study, the bleaching agent was not in direct contact with dentin, but it was spread over the enamel surface. In this case, penetration may occur from enamel to pulp through dentin. Total accumulated bleaching hours for 2 weeks period was 72 hours. The concentration of active hydrogen peroxide in 10% carbamide peroxide is 3.5%. With this low concentration and length of treatment, dentin was not affected significantly. As a result, the alterations in SEM images were not recognizable both in the peritubular and intertubular dentin. The bleached specimens did not show any morphology difference compared to the specimens stored in distilled water.

Vickers hardness test was used as a practical method to detect the surface changes of teeth exposed to the diverse environments. The microhardness of three different cases in Table 1 did not show statistically significant differences. This indicates that bleaching teeth with 10% carbamide peroxide

gel for 6 hours/day for 2 weeks period does not affect the hardness of dentin. The microhardness of a tooth partially indicates the state of elements within the tooth structure. Since bleaching gel easily penetrates the tooth and interacts with the dentin structure, an active 3.5% hydrogen peroxide in 10% carbamide peroxide gel may not be strong enough to change the state of elements in dentin for 2 weeks period.

Fourier transform Raman (FT-Raman) spectroscopy is recognized as a significant analytical method for biomedical applications^{19,20}. The FT-Raman spectrum contains a significant amount of information regarding the composition and structure of specimens at the molecular level. Any change in a tooth will affect the states of molecules and vibrational modes. FT-Raman spectroscopy is able to detect microscopic changes in a tooth, which the microhardness test missed. Fig. 5 may confirm the previous findings in Fig. 1~4 and the result of the microhardness test. The presented FT-Raman spectra did not show any spectral difference. The amide bands on collagen and organic C=H stretching vibrations at 2950 cm^{-1} were not changed after bleaching. Inorganic phases were also unchanged. From this result the dentin structure may not be affected whether the penetrated low concentration carbamide peroxide reacts with internal dentin structure or not. No significant reduction or rising of the Raman peak, which indicates any chemical composition change through the reaction, was observed.

An electron probe microanalyzer equipped with a wavelength dispersive spectrometer (WDS) uses the x-ray wavelength and intensity. The WDS can evaluate the mineral elements with an accuracy of 0.01 wt%²¹. The use of this accurate method is beneficial in the areas where small differences in the elements are expected. The total content of elements in three different cases did not show any statistically significant difference. Insignificant change in mineral content may confirm the previous findings in this study. The change of Ca and P after additional bleaching was negligible. The Mg content remained almost unchanged. Magnesium is known to be preferentially lost during the earliest stages of demineralization²². Since Mg is among the first elements to be dissolved in the demineralization process, this change could be the first sign of demineralization. Insignificant

change of FT-Raman spectra in the inorganic phase may reflect the negligible change of Mg content in dentin during the bleaching process. The change of Zn content reflects the result of tissue degradation. Zn activates enzymes, which are required for the cleavage of collagen²³. The negligible content change in Zn may imply an insignificant reaction of penetrated bleaching agent with dentin.

From the qualitative and quantitative analysis, the effect of an additional bleaching of teeth with 10% carbamide peroxide gel was found to insignificantly affect the structure of dentin. The results may confirm the safety of using 10% carbamide peroxide gel for 2 weeks period on dentin.

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Abstract

EFFECT OF 10% CARBAMIDE PEROXIDE ON DENTIN

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The teeth bleaching with bleaching agent is widely used at recent times. Until yet the exact mechanism of the bleaching agent isn't known but it is thought that is by the complex reduction-oxidation reaction of the decomposed free radical from bleaching agent through various ways. In other words, it is supposed that the teeth are whitened by agent's changing chemical structures of stain-causing materials. The purpose of this study is to examine the change of the dentinal character by bleaching agent and to evaluate the safety of this agent. For this study, after applying 10% carbamide peroxide to enamel of human premolar for 6 hours a day for 2 weeks we examined changes of surface morphology, microhardness, composition and contents of minerals in human dentin using SEM, microhardness tester, FT-Raman spectrometer and EPMA and got following results.

There was no significant difference in surface morphologic change when we examined the effect of 10% carbamide peroxide which penetrated into dentin after applied on enamel surface comparing with result from specimen in distilled water. No change was shown on the surface of peritubular and intertubular dentin within the nanometeric range. The microhardness between bleached teeth and teeth stored in distilled water showed no statistically significant difference. FT-Raman spectra of dentin exhibited no change of the component in human dentin. Only the least change in peaks of organic and inorganic materials were detected in Raman intensity. The total content of mineral elements in dentin with no treatment, stored only in distilled water and stored in distilled water after bleaching were 98.73 ± 1.89 , 98.56 ± 2.11 and 97.47 ± 2.51 respectively. Also they showed no statistically significant difference.

From above results, the effect of 10% carbamide peroxide bleaching on structure of dentin is very low and the results may confirm the safety of this bleaching agent.

Key words : Bleaching, 10% carbamide peroxide, Dentin, FT-Raman spectrometer, Microhardness