The Effects of Tooth Bleaching Agents on Microhardness of Enamel *in situ*

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ABSTRACT

The objective of this *in situ* study was to evaluate the effects of whitening strip (Claren, LG Household & Health Care Ltd, 2.6% hydrogen peroxide) and gel (Opalescence, Ultradent, 10% carbamide peroxide) on microhardness of enamel in comparison with untreated control. Extracted twenty human upper incisors were disinfected, cleaned, and labial side of each incisor sectioned into 3 fragments by 2 × 2 ㎟ size. After sectioning, labial sides of fragments were flattened and fixed to orthodontic bracket using flowable composite resin. Specimens prepared from each tooth were attached to the labial side of upper incisors of twenty volunteers one by one and treated by three different methods: (1) untreated control (2) treated with whitening strip for 14 days (3) treated with whitening gel for 14 days.

Microhardness (Microhardness tester, Zwick) of each specimen was measured at the baseline of pre-treatment, immediate after bleaching treatment, 14 days after bleaching treatment and Knoop Hardness Number was determined. Microhardness changes of experimental groups were compared.

The results show that tooth whitening strip and gel used in this study does not effect the microhardness of enamel during bleaching procedure. (J Kor Acad Cons Dent 31(6):470-476, 2006)

Key words: Whitening strip, Tooth whitening gel, Enamel, Microhardness

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Ⅰ. INTRODUCTION

The demand for conservative esthetic dentistry has dramatically grown and so has the rapid development of new non-restorative treatments for discolored teeth[1]. Nightguard vital bleaching has become one of the most frequently used treatment modality for improving the esthetic appearance of teeth, primarily because of its relative ease of application, the safety of 10% carbamide peroxide bleaching materials, reduced patient chair time, lower cost, and high percentage of success[2]. The recent development of a strip-based system allows for vital tooth bleaching without fabrication of a custom tray. Applied directly to the tooth surface, this thin, flexible polyethylene strip coated with a hydrogen peroxide bleaching gel may afford some in-use advantages relative to tray-based systems[3].

Carbamide peroxide is usually used at a concentration of 10% to 15% in dental bleaching. 10% carbamide peroxide is equivalent in strength to 3% hydrogen peroxide. The hydrogen peroxide,

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because of its instability and ease of decomposition into water and oxygen, penetrates through the pores of enamel and dentin to provide the lightening of the teeth. Bleaching agents affect the lightening of tooth structure through decomposition of peroxides into free radicals. The free radicals break down large pigmented molecules in enamel and dentin into smaller, less pigmented molecules[4]. If a prolonged contact time is spent, protein matrix of enamel and dentin might be broken. Therefore, one possible side-effect of bleaching products is that the enamel and dentin may be weakened by oxidation of the organic or inorganic elements[4].

Whitening strips are being used more and more commonly at home. However, there was no previous studies on side-effects to enamel bleached with commercially available whitening strips.

The purpose of this *in situ* study was to evaluate the effects of whitening strip and gel on the microhardness of enamel in comparison with untreated control *in situ*.

Ⅱ. MATERIALS AND METHODS

Materials

Commercially avaliable whitening strip and bleaching gel were used in this study: Claren dental whitening solution (LG Household & Health Care, Seoul, Korea) and Opalescence tooth whitening gel (Ultradent Product Inc, South Jordan, USA). Claren dental whitening solution is strip type and has the hydrogen peroxide at the concentration of 2.6%. The Opalescence bleaching gel used in nightguard bleaching is composed of 10% carbamide peroxide with carbopol.

Selection of Volunteers

The volunteers were 20 adults (15 males and 5 females) from 24 to 35 years of age. Each volunteer was informed of the objectives, benefits and possible risks involved in this experiment and participated only after providing written formal consent of Institutional Review Board of Kangnung National University Dental Hospital. Exclusion criteria for participating in this study were who has fixed or removable dentures or orthodontic appliances, pregnant or nursing women, smokers and had dentin sensitivity. All volunteers were used same tooth brush & toothpastes for experimental period.

Test sample fabrication

We used freshly extracted twenty upper incisors containing no apparent evidence of cracks, restorations, fracture, cervical abrasion and caries for this study. Teeth were disinfected, cleaned, and labial side of each incisor sectioned into 3 fragments by 2 × 2 ㎜ size using precision cutting device (Acutom P-50, Struers, Copenhagen, Denmark). After sectioning, the labial sides of fragments were flattened and fixed to orthodontic bracket using flowable composite resin (FiltekFlow, 3M ESPE, St. Paul, USA) and stored in distilled water until used. Before fixed to orthodontic brackets, specimens were examined under surgical microscope (OPMI, Carl Zeiss, GmbH, Germany) for checking cracks or fractures of enamel.

Three orthodontic brackets with a dental fragment from one tooth were randomly fixed to the central labial surface of the maxillary incisors using Glass ionomer luting cement (Rely-X luting, 3M ESPE, St. Paul, USA). Bleaching gel group (Group C) was attached to the maxillary right central incisor, control group (Group G) to the maxillary right lateral incisor, bleaching strip group (Group S) to the maxillary left central incisor (Table 1).

Bleaching procedure

1) Group C (Control)

Brackets were remained to tooth without bleaching treatment for 14 days.

2) Group G (Opalescence tooth whitening gel)

Orthodontic brackets were blocked out using utility wax for reservoir of bleaching gel. And

Table 1. Experimental groups

Group	Number of tooth	Bleaching material
C	20	Control (No treatment)
G	20	Opalescence tooth whitening gel (Ultradent Product Inc, South Jordan, USA
S	20	Claren dental whitening strip (LG Household & Health Care, Seoul, Korea)

then impressions were taken with alginate and stone cast models were made. Mouthguard trays were fabricated for each volunteer in a vacuum-forming machine using a flexible ethyl vinyl acetate polymer that was 0.4 ㎜ thick. Bleaching was performed for 2 hours per day for 14 days using fabricated custom tray.

3) Group S (Claren dental whitening solution)

Bleaching was performed with corresponding whitening strips for 30 minutes 2 times per day for a period of 14 days.

Microhardness tests

Microhardness test was performed using a Hardness testing machine (Microhardness tester, Zwick, Ulm, Germany) and TestXpert program (Zwick, Ulm, Germany). Three indentations on each specimen were made with a load of 300 g for 25 seconds dwell times.

Microhardness were measured at three different periods.

1) Before bleaching treatment

Microhardness measurement were performed before brackets were attached to volunteers.

2) After bleaching treatment

After 14 days of *in situ* bleaching treatments, brackets were removed from volunteers and were again tested for microhardness. After microhardness measurement, the brackets were reattached to volunteer.

3) After remineralization

The specimens were kept in oral cavity for 14 days for remineralization and then brackets were removed from volunteers and microhardness measurements were performed.

Statistical analysis

Microhardness changes were statistically analyzed and compared between groups using Repeated measure of ANOVA and Scheffe post-hoc test at the 95% level of confidence. One way-ANOVA and Scheffe post-hoc test was used to compare differences for degree of microhardness change between groups at the 95% confidence level.

Ⅲ. RESULTS

The mean values and standard deviations of experimental groups at initial, after bleaching and after remineralization are shown in Table 2. Although the mean microhardness values for all groups were increased after 14 days, they decreased after remineralization (Figure 1). There was no difference in the microhardness between experimental groups at all measurement time.

Table 3 shows that the mean and standard deviations of the changes in the microhardness (After bleaching-initial, after remineralization-after bleaching) of experimental groups. There was no difference between experimental groups.

Table 2. Mean microhardness values and standard deviations of experimental groups (Mean ± SD, Unit: VHN)

	Group C	Group G	Group S
Initial	276.97 ± 70.06	308.32 ± 69.05	282.28 ± 78.11
After bleaching	283.86 ± 69.51	319.21 ± 71.37	283.65 ± 76.01
After remineralization	282.98 ± 69.73	315.93 ± 72.54	280.40 ± 77.19

Table 3. The amount of change in microhardness (Mean ± SD, Unit: VHN)

	Group C	Group G	Group S
After bleaching - Initial	6.89 ± 18.75	10.88 ± 12.48	1.37 ± 17.68
After remineralization - After bleaching	-0.88 ± 8.12	-3.28 ± 12.15	-3.25 ± 10.26

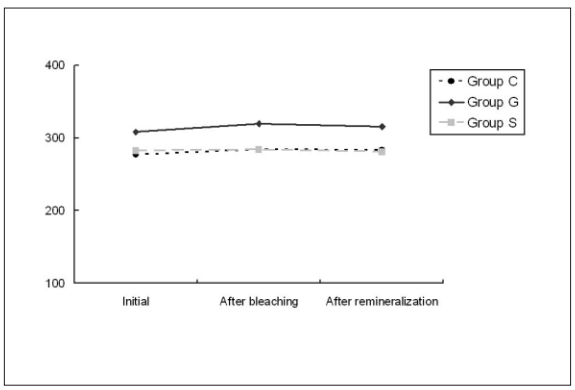

Figure 1. Microhardness changes (Unit: VHN).

Ⅳ. DISCUSSION

Currently, the general population relates modern dentistry to improved facial aesthetics, health, and social success. A lighter dentition is associated with health, youth, and vigor. A recent survey of American women showed that 55% of those between 34 and 55 years would have their teeth whitened or straightened to create a more youthful appearance[5]. Tooth whitening is now the most commonly requested elective cosmetic service in the dental office.

The recent development of a strip-based system allows for vital tooth bleaching without fabrication of a custom tray. Previous research on whitening strips had demonstrated that twice daily treatment over 14 day period resulted in a meaningful improvement in tooth color relative to baseline and placebo[6]. The magnitude of the benefit was similar to that achieved using a conventional 10% carbamide peroxide system in a custom tray, and superior to that seen with a 10% combination carbamide peroxide system in a stock tray[7].

One of the possible side effects of bleaching products is that the enamel structure may be weakened by oxidation of the organic or inorganic elements[4]. Various test methods, such as SEM, AFM have been used to evaluated the changes in enamel surface during bleaching. In this study, effect to bleaching was assessed by the microhardness changes. Microhardness changes are related to a loss or gain of mineral (demineralization or remineralization) of the tooth structure[8]. It has been shown that the microhardness test is suitable for determining small changes in surface microhardness that demonstrate the effects of acids, acidic beverages and bleaching products on enamel[9-11]. Morphological characterization by SEM is a good complement to microhardness measures in offering a more complete understanding of tooth surface conditions following bleaching exposure[12].

For bleached enamel surfaces, there was a tendency of reduction of the microhardness of either bleaching agents compared to the unbleached ones[13-15]. However, this study indicated no statistically significant differences in microhardness changes between groups. Using similar methodology to evaluate the microhardness after 10% carbamide peroxide treatment on enamel, Justino *et al*[16] and other investigators[17-18] found similar results. Conflicting results have been obtained in studies evaluating the adverse effects of bleaching treatment. It is possible that discrepancies found in these studies can be explained by variables in methodology related to exposure time, pH of solution, type of teeth, and storage medium. In that regard, the storage medium seems to be an important factor for such variation in results. In the study of Justino *et al*[16], difference between the *in situ* and *in vitro* condition could be attributed to the important role of human saliva in the remineralization process and while enamel slabs from the *in vitro* methodology were stored in deionized water, slabs from the *in situ* group were submitted to a clinical condition in oral appliances used four volunteers. The presence of saliva could prevent the demineralizing effect of bleaching gel *in situ*.

There is a correlation between the values of microhardness evaluations allowed by the dynamics of the oral cavity. Demineralization of enamel structure occurs at a critical pH of 5.5[19]. In a pH of less than 5.5 the amount of Ca and P in saliva is lower than the solubility rate of hydroxyapatite, with enamel having a tendency to lose Ca and P to the oral environment[16]. When the bleaching agent causes demineralization in enamel, ionic changes are induced, increasing mineral uptake, which replaces the mineral lost during treatment. In addition to, because bleaching is a time-and dose-related treatment, it is conceivable that the effect of bleaching treatment on human enamel is directly related to the bleaching agent's concentration of peroxide and the contact time[13]. In this study, 10% carbamide peroxide and 2.6% hydrogen peroxide strip were used to bleaching only for 2 weeks, so it can be thought that there was relatively short bleaching period and low concentration of bleaching agent compared to other studies.

In this study, mean microhardness values for all groups increased after 14 days, where as microhardness decreased after remineralization for experimental period (Figure 1). A possible cause for this pattern might have been the storage medium used during the preparation of the enamel fragments, namely distilled and deionized water. Araujo *et al*[18] have observed in pilot studies that when enamel is immersed in deionized water for long periods of time, microhardness values drop. A few studies have shown that demineralization enamel is more susceptible to remineralization than intact enamel[20]. Thus, in spite of bleaching agents having a slightly acidic pH, the solution is rapidly neutralized, which cancels its demineralization potential[21]. According to Leonard *et al*[21], 10% carbamide peroxide significantly increases salivary pH during the first 15 to 20 minute interval. This saliva neutralization is apparently related to the breaking down of carbamide peroxide into urea, whereby the pH is raised. This might explain the slight increase in microhardness observed to occur in this study.

In the laboratory it is difficult to duplicate the clinical conditions completely in which bleaching treatment is conducted. Therefore, it may not be entirely possible to extrapolate the results of *in vitro* studies to the clinical setting. In the study of Araujo *et al*[18], they used removable appliance to replicate closely the intraoral conditions. But several limitations and variables have to be considered. The appliances containing the enamel specimens were removed from the mouth during meals and toothbrushing. This was necessary to avoid the contact of the specimens with chemicals that could affect enamel hardness. In this study, enamel fragments were fixed on the upper incisors of 20 volunteers during experimental period except microhardness test procedure.

The baseline values obtained in our study are in agreement with the mean microhardness values established by others[14-18].

Ⅴ. CONCLUSION

In this study, microhardness to enamel bleached with commercially available bleaching agents were evaluated *in situ*. Although the mean microhardness values for all groups increased after 14 days, where as microhardness decreased after remineralization, bleached groups did not show significant differences from untreated control group in tendency and amount of microhardness changes ($p > 0.05$).

Therefore, it can be stated that bleaching with whitening strips and the bleaching gel did not influence the microhardness to enamel within 14 days.

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국문초록

수종 치아미백제가 구강내에서 법랑질의 미세경도에 미치는 영향

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본 연구는 치아미백용 2.6% hydrogen peroxide 부착대와 통상 home bleaching용으로 안전하다고 알려진 10% carbamide peroxide 미백젤이 법랑질 미세경도에 미치는 영향을 미백을 하지 않은 경우와 비교, 평가하기 위하여 시행되었다.

최근에 발거한 사람의 상악 절치 20개를 사용하여 가로, 세로 2 ㎜의 정사각형 치아편을 각 치아에서 3개씩 절단, 채득한 후 교정용 bracket에 고정하여 시편을 제작하였다. 자원자는 20명을 선정하였고, 각각의 자원자의 치아에 동일 치아에서 제작한 시편이 부착된 bracket을 순면 중앙에 부착하였으며 상악 우측 측절치에 젤을 이용한 군 (Opalescence tooth whitening gel, Ultradent Product Inc, South Jordan, USA), 상악 우측 중절치에 미백을 하지 않은 대조군, 상악 좌측 중절치에 부착대를 이용한 군 (Claren, LG Household & Health Care, Seoul, Korea)을 부착 시켰다. 젤을 이용한 군은 bracket이 부착된 치아에 맞게 상악 우측 측절치부터 우측 소구치까지를 덮는 mouthguard tray를 제작하여 매일 동일시간에 2시간씩 14일 동안 미백을 시행하였으며, 대조군은 부착 28일 동안 미백 치료 없이 구강 내에 위치시켰다. 부착대를 이용한 군은 bracket의 시편을 덮을 수 있는 크기로 상악용 Claren을 절단하여 오전, 오후 각각 30분씩 하루 두 번 부착하여 14일 동안 미백을 시행하였다. 각 군 모두 bracket 부착 전, 미백 14일 후, 재광화 14일 후의 미세경도를 측정하였으며 측정된 값을 이용하여 미세경도 변화 양상과 변화량을 비교 평가하였다.

치아미백용 2.6% hydrogen peroxide 부착대와 젤 형태의 10% carbamide peroxide 미백제의 미백 전, 미백 후, 재광화 후 미세경도 변화 양상이 미백을 하지 않은 대조군과 차이를 보이지 않았으며 ($p > 0.05$), 미백 전과 미백 후의 미세경도의 차이, 미백후와 재광화 후의 미세경도의 차이도 유의할 만한 차이가 없었다 ($p > 0.05$). 따라서 시중에 판매되고 있는 whitening strip과 미백 젤은 14일 동안의 통상적인 미백과정 동안 법랑질의 미세경도에 영향을 미치지 않는 것으로 사료된다.

주요어: Whitening strip, 미백젤, 법랑질, 미세경도