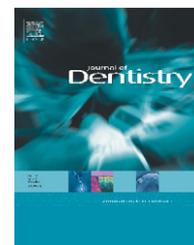


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Effect of laser irradiation on crystalline structure of enamel surface during whitening treatment with hydrogen peroxide

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ABSTRACT

Objective: This study is to evaluate the effect of laser activation on the whitening and crystalline structure of enamel surface during whitening treatment with hydrogen peroxide. **Methods:** Bovine teeth were treated with whitening gel containing 35% hydrogen peroxide. A whitening gel was applied on the enamel surface for a period of 5 min, and then irradiated using a diode laser (740 nm) during whitening treatment for 0, 30, 60, 120 and 180 s for the GL0-W, GL30-W, GL60-W, GL120-W and GL180-W groups, respectively. The total whitening application time was 30 min for all groups.

Results: Laser-irradiated enamel groups showed a similar lightness compared to the GL0-W group. The thickness of porous layer observed on the enamel surface of GL0-W group was decreased by increasing the laser irradiation time. While the Ca and P contents of the GL0-W group were lower than those of the non-whitening treated group (GL0-C), the Ca and P contents of the GL180-W group were similar to those of the GL180-C group. The enamel crystallinity was dramatically decreased by whitening treatment without laser irradiation. However, the decrease of crystallinity was protected by laser irradiation during whitening treatment. Raman measurement verified that laser irradiation could prevent the loss of mineral compositions on enamel and maintain its crystalline structure.

Significance: The professional whitening treatment with hydrogen peroxide and diode laser activation improves not only the whitening effect but also protects the change of enamel structure compared to the treatment with only gel.

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1. Introduction

Tooth whitening is a cosmetic treatment which can be performed on non-vital and vital teeth using various concentrations of hydrogen peroxide (3–38%) and carbamide

peroxide (10–30%). Tooth whitening can be categorized into two types: self (at home) whitening and professional (in-office) whitening. Professional whitening treatment is performed by using a high concentration of hydrogen peroxide (35–38%), while activating agents such as heat, light or laser increase the efficacy of the whitening treatment.^{1–5}

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The first example of professional bleaching of stained teeth was that accomplished by M'Quillen in 1867.⁶ In 1937, Ames applied a heat source to 35% hydrogen peroxide.¹ In 1989 tooth whitening became largely used with the introduction of a whitening gel by Haywood and Heymann.⁷ Since the early 1980s, a heated spatula or a heat lamp has been used as a heat source to accelerate the bleaching process of concentrated hydrogen peroxide. As bleaching techniques developed, several different types of irradiation sources such as light, LED or laser have been used to accelerate the professional bleaching procedure.²⁻⁴ Among irradiation sources, laser tooth bleaching began in 1996, with the approval of the argon laser (480 nm) and the CO₂ laser (10.6 μm).⁵ The advantages of professional bleaching over home bleaching include dentist control, avoidance of soft-tissue exposure and reduced total treatment time.

Generally, dental bleaching is accompanied by increased tooth or gingival sensitivity.⁸ Also, some evaluations have reported that the microhardness value and calcium content of enamel decrease and its surface micromorphology changes after exposure to peroxide based formulations.⁹⁻¹³ As mentioned above, many studies have evaluated the effects of peroxide-based products on the physical and chemical properties of tooth enamel.^{11,13,14} If additional laser irradiation during whitening-gel treatment improves tooth bleaching, the morphology and/or the crystalline structure of enamels could be different between laser-treated and non-treated teeth. However, there are insufficient studies about how enamel crystalline structures are affected by laser irradiation during whitening gel treatment. In this study, therefore, the effect of laser activation on the whitening and crystalline structure of enamel surface during whitening treatment with hydrogen peroxide was investigated.

2. Materials and method

2.1. Tooth preparation

Twenty bovine teeth were used for this study. Bovine tooth is convenient to evaluate the characteristics enamel surface because they have a relatively large flatness and uniform compositions.¹⁵ The extracted teeth were cleaned and stored in an aqueous solution of 0.1% thymol for over 24 h. After the teeth were washed with distilled water, the crown was separated from the root using diamond precision cutting system (Exakt, DE/300CL/CP, USA) with water. In order to decrease the thickness of teeth specimens to a 3-4 mm, the lingual surface was polished wet using a polishing machine (Buehler Ltd., Metaserv 250, USA) with SiC polishing paper (300 grit).

Since differences occur in the chemical composition and crystalline structure of the enamel on each individual tooth, the five crowns used for surface characterization were divided in half longitudinally in order to effectively analyse the whitening outcomes. Each half tooth divided in this way was kept for each control group, while the other teeth were used in the whitening treatment.

2.2. Whitening treatment

Tooth whitening procedures are shown in Fig. 1. Before the whitening gel was applied on the sample groups, tooth colour and the mean values of $L^*a^*b^*$, were measured using a colorimeter (NF999, Nippon Denshoku, Japan). The whitening gel (SHINY multi-functional polishing gel, Blueberry, Korea) containing 35% hydrogen peroxide was applied on the enamel surface with a thickness of approximately 2 mm in accordance with the manufacturer's instruction. After 5 min, the specimens were irradiated using a diode laser (Diodent II, HOYA combo) with a 740 nm wavelength for 0, 30, 60, 120, and 180 s for the GL0-W, GL30-W, GL60-W, and GL120-W groups, respectively. Laser power was set to 300 mW and the spot size was 6 mm. The total whitening treatment time was 30 min for the sample groups. The gel was then removed and the specimens were washed with water. After 1 h, the tooth colour was measured again. Three teeth and one divided tooth from each group used for whitening treatment and colour measurement. GL0-C, GL30-C, GL60-C, GL120-C, and GL180-C groups were non treated whitening groups, the divided other half teeth of GL0-W, GL30-W, GL60-W, GL120-W, and GL180-W groups, respectively, for scanning electron microscopy, X-ray diffraction, and Raman spectroscopy.

2.3. Surface characteristics

The surface and cross-sectional morphologies of tooth enamel were observed using Scanning Electron Microscope (SEM; Hitachi N-3500, Japan). The specimens were sputter-coated with Au-Pd film in order to avoid an electron charging effect. In order to take cross-sectional images of the enamel, the specimens were embedded using epoxy followed by crosscutting, and then polished using SiC polishing papers (#600) and finally polished with alumina powder (dia. 0.3 μm). Chemical compositions were evaluated using Energy dispersive X-ray spectroscopy (EDX; Horiba EX-250, Japan). The crystalline structure of enamels of whitening-treated and non-treated teeth was analysed by an X-ray diffractometer (XRD; PANalytical, X'Pert PRO, Netherlands) with Cu K α radiation (30 mA, 40 kV) at a scan speed of 0.067°/s from 10° to 80°. The merged XRD peaks were de-convoluted in order to calculate intensity

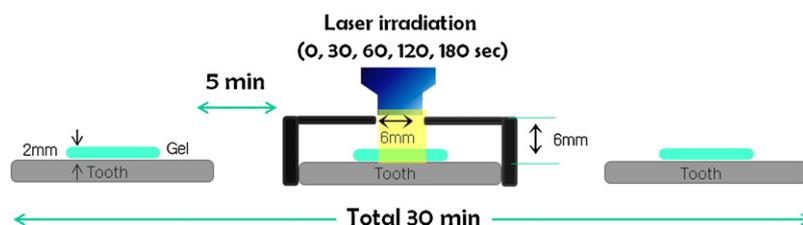


Fig. 1 – Schematic diagram of laser irradiation procedures while whitening treatment of tooth enamel.

and FWHM (full width maximum height) using OriginPro software (OriginLab, OriginPro 8.5, USA). Raman spectroscopy (Renishaw, inVia Raman microscope, UK) was used to analyse the inorganic compositions in enamel. For the GL180-C and GL180-W groups, the enamel crystalline structures were examined by high resolution transmission electron microscopy (HRTEM; Techni F20, Philips, Netherlands) operating at 200 kV. The TEM specimens were prepared by cutting the enamel in a cross-sectional direction using a focused ion beam (FIB; FEI Co., Quanta 3D, Netherlands).

2.4. Statistical analysis

The data were analysed using OriginPro software (OriginLab, OriginPro 8.5, USA). A one-way ANOVA followed by a Tukey post hoc test was used to determine group differences and effects. $P < 0.05$ was considered to be significant.

3. Results

Fig. 2 shows the discrepancy between the lightness (ΔL^*) on teeth before and after the whitening treatments. The results indicate a significant improvement in brightness through the whitening treatment. While the mean value of changes in lightness for whitening-treated teeth combined with laser irradiations were higher than that for the only gel-treated tooth, but it was not significant.

Fig. 3 shows the XRD patterns for the control group and whitening treated sample groups. All XRD peaks were attributed to a hydroxyapatite structure. The intensity of the (002) peak was dramatically decreased for the GL0-W group, in which only gel used in the whitening treatment. However, it was increased as the laser illuminating time increased when it was compared to the intensity of GL0-W group. Fig. 5 showed that the (002) peak intensity was apparently dependent on the laser irradiation time. The XRD peaks observed at approximately 32° were separated according to (211), (112) and (300) XRD peaks by the Gaussian

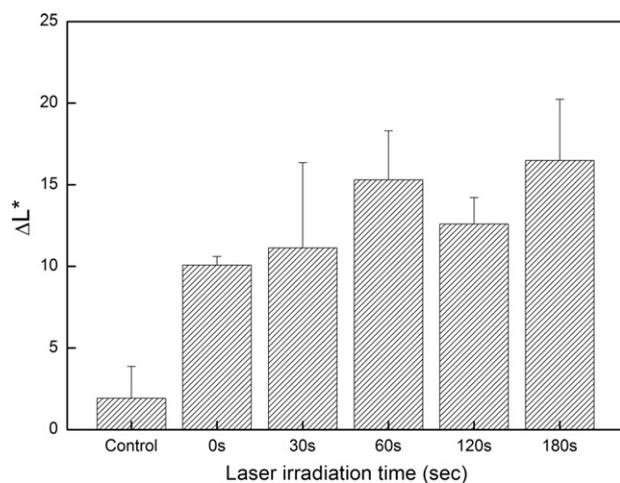


Fig. 2 – Discrepancy of brightness on teeth before whitening treatment and after 1 h of the whitening treatment.

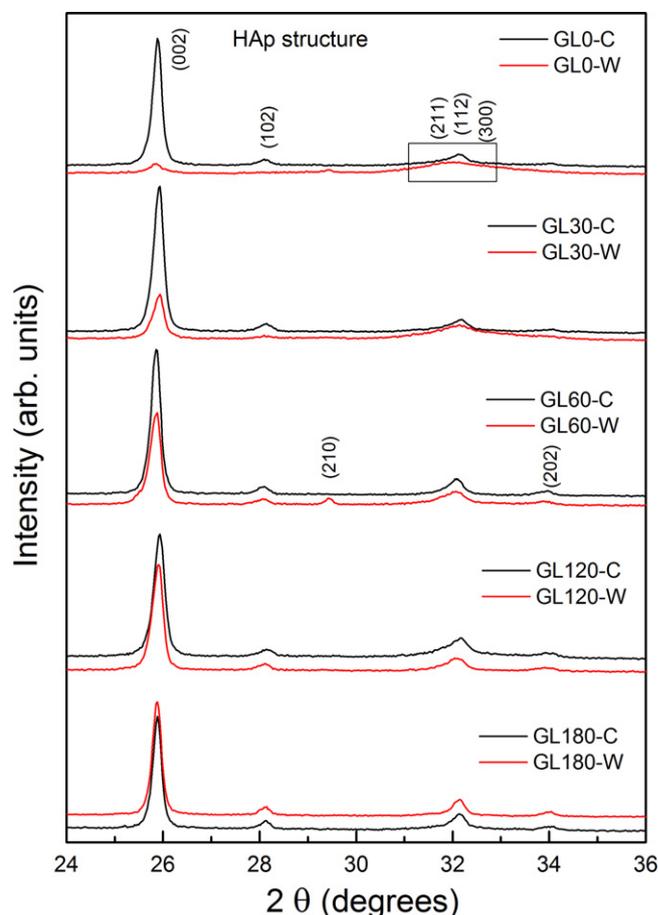


Fig. 3 – XRD patterns for whitening treated sample groups and each control groups.

deconvolution method for the GL0-C, GL0-W, GL180-C and GL180-W groups (Fig. 4). After whitening gel treatment (GL0-W), the full width half maximum (FWHM) of the (112) peak was broader and the intensity of the (300) peak was higher than those of the control group (GL0-C). However, the GL180-W group (whitening treated using gel and laser irradiation) showed that the FWHM of the (112) peak was similar to that of the GL180-C group. Fig. 5 shows that the FWHM (112) peak was decreased for the GL120-W group.

Fig. 6 shows the Raman spectra for the whitening treated groups and control groups. Although the P–O peaks ($440, 579, 960, 1071 \text{ cm}^{-1}$) originating from hydroxyapatite were dominant for the GL0-C group, the GL0-W group showed that the P–O peaks were weakened and amidic ($1452, 1675 \text{ cm}^{-1}$) and the C–H ($2881, 2940 \text{ cm}^{-1}$) peaks caused by organic matters such as collagens were prominent. As laser irradiation time increased, the peaks related to organic materials eminently smoothed out and P–O peaks again were observed dominantly.

As seen in Fig. 7, the deep cracks were located in only the whitening gel-treated GL0-W group. However, the surface morphology of the GL180-W group irradiated with a laser for 180 s did not show significant difference from that of the GL180-C group. In addition, the analyses of chemical compositions using showed that the inorganic composition of Ca and P

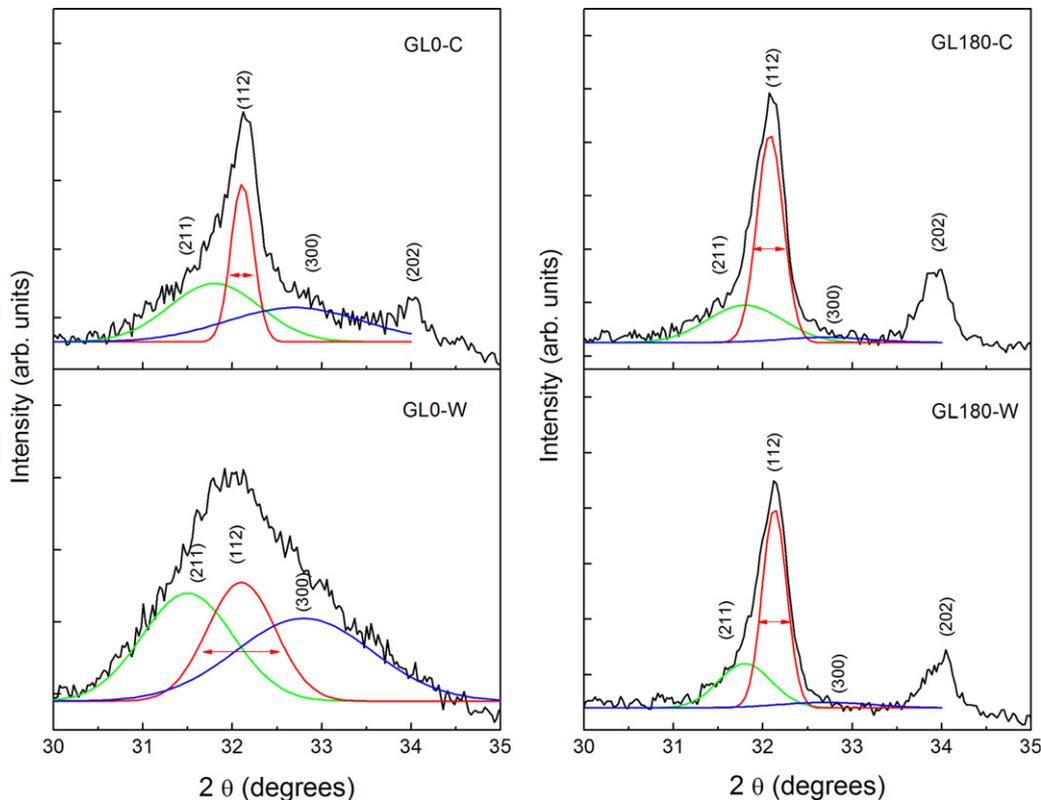


Fig. 4 – (2 1 1), (1 1 2) and (3 0 0) XRD peaks divided by Gaussian deconvolution method for GL0-C, GL0-W, GL180-C and GL180-W groups.

decreased and the organic composition of C increase for GL0-W group in comparison to GL0-C group. However, the GL180-W group showed little change in chemical composition compared to the GL180-C group.

Fig. 8 shows cross-sectional SEM images of the enamel layers for each group. The new porous layers with 200–250 μm thickness above the enamel surface were observed for the

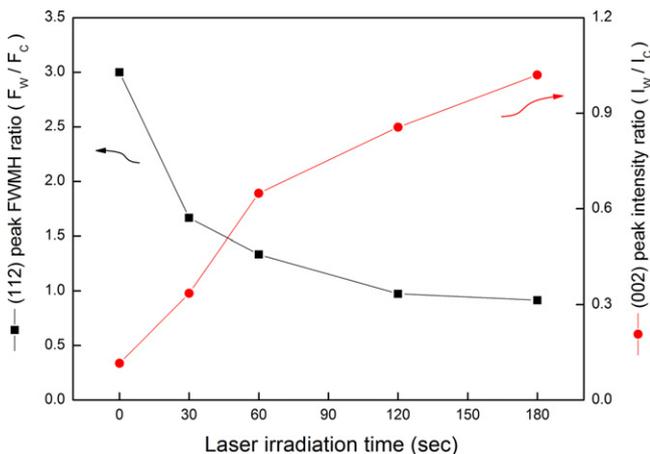


Fig. 5 – Changes of (0 0 2) peak intensity and FWHM values of (1 1 2) peak of whitening treated teeth groups compared with those of control groups according to the laser irradiation time.

GL0-W and GL30-W groups. The chemical composition of the new layers indicated a relatively smaller portion of Ca and P in comparison to that of the sound enamel region. The thickness of the new porous layer for GL60-W group was decrease with increasing laser irradiation time. There was no sign of an extra layer besides the sound enamel layer in the GL180-W groups.

Fig. 9 shows the TEM images and selected area electron diffraction (SAED) patterns of the GL180-W and GL180-C groups. Well-arranged enamel rods (region A) were observed from the surface in the GL180-C group. The new phase with 0.5–1 μm thickness (region C) was observed on the tooth surface for the GL180-W group. This phase was not observed in the cross-sectional SEM image (Fig. 7) due to its low thickness. The region B of the GL180-W group was similar to that of the GL180-C group. This result indicated that the arrangement of enamel rods was destroyed by the whitening treatment of the tooth.

4. Discussion

The recent development of laser-assisted tooth whitening procedures offers patients an easier, faster, non-invasive, and affordable way to have whiter teeth.^{16–18} The objective of laser bleaching is to achieve the ultimate power bleaching process using the most effective energy source, while avoiding any adverse effect.¹⁹ A laser beam can activate high concentration hydrogen peroxide (35%) extremely quickly and thus help to

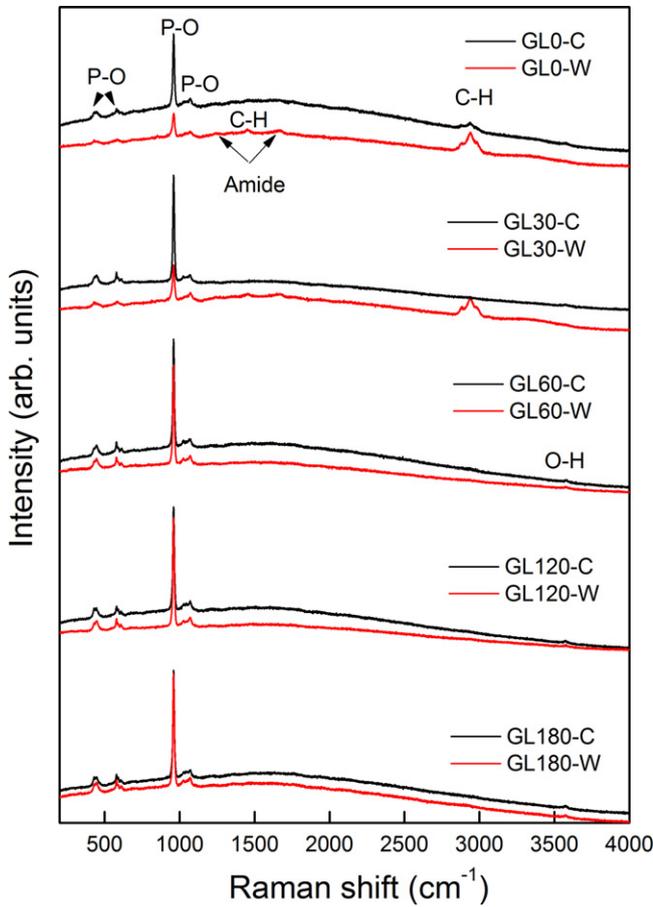


Fig. 6 – Raman spectra for whitening treated groups and control groups.

achieve satisfactory whitening of teeth and diode lasers have a greater penetration depth compared with other laser systems. This study also demonstrates that laser radiation was clearly influential in tooth whitening as shown in Fig. 2. Although laser irradiation is known to be an effective method to improve tooth whitening, it is important to investigate this effect on the change of enamel structure and compositions because tooth whitening using high concentration peroxide has adverse effects such as tooth or gingival sensitivity.^{20,21} In this study, surface and cross-sectional images, the crystalline structure and chemical compositions of enamel whitening-treated combined with laser irradiation were evaluated using various material characterization methods such as SEM, XRD, EDX, Raman, and TEM.

The XRD peak of the (0 0 2) plane of the HAp structure is observed as the main peak in the XRD patterns of the tooth surface due to the enamel microstructure^{22,23} which has the c-axis oriented arrangement of the enamel rods, while that of the (2 1 1) plane is observed as the main XRD peak for the chemically synthesized HAp.²⁴ The GL0-C group showed typical XRD patterns of tooth enamel structure as can be seen in Fig. 3. However, XRD diffraction patterns of the GL0-W group showed an acutely decreased (0 0 2) peak. This result indicated that the tooth enamel structure was dramatically changed after whitening treatment. The FWHM value of the XRD peak is dependent on the crystallinity of the measured specimen. If the peak width is narrow, the crystallinity of the specimen is high. Therefore, the increase of FWHM value of XRD peak (1 1 2) for GL0-W indicated that the crystallinity of enamel was decreased by whitening treatment.

It has been reported that these outcomes can be observed when using hydrogen peroxide in the tooth whitening process:

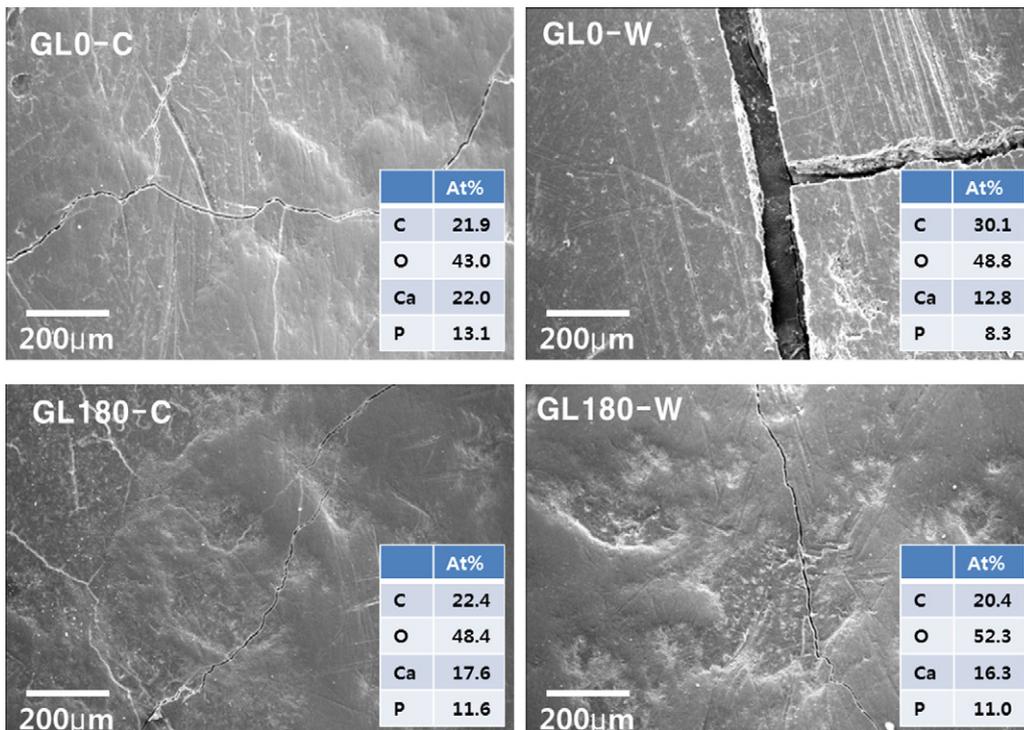


Fig. 7 – Surface morphologies and chemical compositions of whitening-treated GL0-W and GL180-W groups and their control groups.

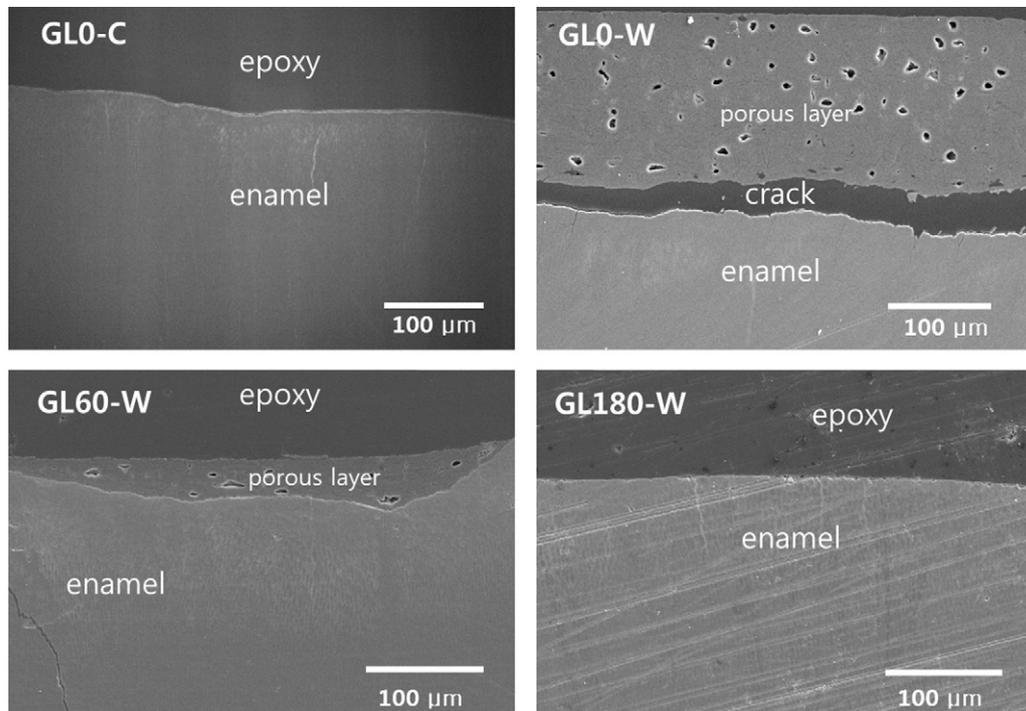


Fig. 8 – Cross-sectional SEM images of enamel layers for GL0-C, GL0-W, GL60-W and GL180-W groups.

demineralization of the enamel surface of the tooth, significant decrease in the Ca/P ratio of calcium content, and an increase in the porosity of enamel.^{13,25} In this study, the Raman spectrum of GL0-W clearly showed an increase in the organic component and a decrease in the apatite component (Fig. 6). Also, according to EDX analysis, there were predominantly decreased proportions of inorganic materials, calcium and phosphorus. This decrease of inorganic materials might cause cracks to appear on the enamel surface of the GL0-W group, as shown in Fig. 7, because the organic matters had shrunk as the specimen had dried. Fig. 8 shows the demineralized enamel surface, which has a porosity layer of 250 μm maximum thickness for GL0-W group. These results indicated that the structure of the enamel rods in surface was destroyed due to the whitening process.

However, the XRD results in Fig. 2 delineate the lineally increasing tendency of the c-axial (0 0 2) XRD peak according to the increase in the laser irradiation time in comparison to that of the control group. This means that the arrangement of the enamel rod in the c-axial direction does not break down and thus is maintained or improved by being exposed to laser radiation. Furthermore, the FWHM value of the (1 1 2) peak was also similarly restored with the control at 120 s laser irradiation, maintaining the crystalline structure of the enamel. These results lead to the conclusion that the damage of tooth structure occurred during the whitening process using hydrogen peroxide significantly can be reduced by LED laser exposure.

In Fig. 6, the P-O peaks caused by the apatite structure are shown dominantly in the Raman spectra at the laser irradiated

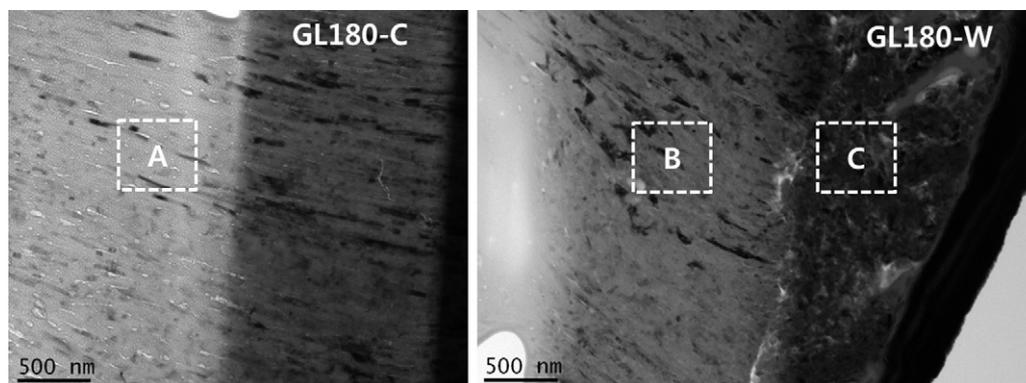


Fig. 9 – TEM images of GL180-W and GL180-C groups. Well-arranged enamel rods are observed on region A and B. The destroyed enamel layer is observed on region C.

groups. A thick demineralized porous layer was observed in the cross-sectional image of enamel treated with only whitening gel. However, SEM images of the cross-section of enamel showed a decrease in thickness of demineralized enamel layer due to the whitening process according to the increase in laser irradiation time. These results clearly demonstrate that laser irradiation during the whitening process not only improves brightness of a tooth but also prevents enamel structure from deformation.

Some assumptions can be made about the phenomenon of why only 3 min of laser irradiation allows enamel structure to be maintained after a total 30 min whitening process. Firstly, a protective layer was made that could obstruct the invasion of hydrogen peroxide into the enamel surface. Although observation on SEM in GL180-W did not show any destroyed enamel layer, a new phase layer with a thickness of less than 2 μm was found in the TEM observation. This phase might have provided a protective layer. Secondly, the chemical property of whitening gel could have been changed through the exposure to laser irradiation. If this assumption is true, it may also be assumed that hydrogen peroxide invades into the enamel surface only at the earlier stage and that hydrogen peroxide no longer enters into the enamel surface due to the change in property of the whitening gel.

Bovine enamel contains significantly more inter-prismatic organic material compared to human enamel even though its structure and compositions are very similar to those of human enamel.²⁶ Therefore, if human teeth are used as specimens, the aspect of demineralization of enamel surface could be different with the results of this study. Further studies are required in order to be more precise about the actual cause.

5. Conclusion

Bovine teeth were treated with whitening gel containing 35% hydrogen peroxide combined with diode laser irradiation. Teeth brightness was increased by laser-assisted whitening. Cracks and pores observed in the enamel of the group treated only with whitening gel (GLO-W) were decreased by increasing the laser irradiation time. While the GLO-W group had low Ca and P contents compared with the non-whitening treated group (GLO-C), the Ca and P contents of the GL180-W group were similar to those of the GL180-C group. The enamel crystallinity was dramatically decreased by whitening treatment without laser irradiation. However, it increased as laser irradiation time increased. In conclusion, professional whitening treatment with hydrogen peroxide combined with diode laser irradiation improves not only the whitening effect but also protect the change of enamel structure compared with the whitening treatment without laser irradiation.

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