

Original

Changes in Dental Enamel Crystals by Bleaching

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Abstract: In recent years, enamel bleaching has been widely conducted clinically. Bleaching involves degrading pigments that are organic substances, and has been thought to have no effect on inorganic substances. However, no study has examined in detail how the enamel changes by bleaching. In the present study, the effects of two commercial bleaching agents; Hi-Lite (Hi-L) and Nite White Excel (NWE), on enamel were investigated by observations with contact microradiography (CMR), scanning electron microscopy (SEM), micro X ray diffractometer (XRD) and high-resolution transmission electron microscopy (HR-TEM), focusing on the effects on enamel crystals. CMR showed no change in degree of mineralization after bleaching with Hi-L or NWE as compared with the control. On SEM, gaps along the prism sheath and cracks between crystals were observed on the enamel surface of Hi-L-bleached and NWE-bleached enamel. XRD demonstrated no remarkable differences in crystal composition and crystallinity after bleaching by both agents as compared with the control. On HR-TEM, crystal growth findings were observed in some regions of Hi-L-bleached enamel, while crystal demineralization findings were observed in NWE-bleached enamel. All the above changes were limited to the very superficial layer of enamel in all the samples.

Key words: Bleaching, Enamel crystals, Hi-Lite, Nite White Excel

Introduction

In recent years, with more and more people interested in cosmetic enhancement, the demand of tooth bleaching is increasing sharply. Not only conventional bleaching of non-vital teeth, the needs for bleaching of vital teeth is also on the increase.

Currently two methods of vital tooth bleaching are available: office bleaching which is conducted by a dentist at the clinic^{1,2}, and home bleaching which is done by the patient at home under the guidance of a dentist³. For office bleaching, Hi-Lite (Shofu, Kyoto) is most popularly used. Hi-Lite is a bleaching agent developed in 1991, and consists of 35% hydrogen peroxide solution as the major ingredient. Through two types of reactions; chemical activation by a catalyst and light activation, the main ingredient is broken down to liberate hydroxyl radicals that has a bleaching action. For home bleaching, Nite White Excel (Discus Dental, USA) is commonly used. This bleaching system was published by Haywood et al³. in 1989. A bleaching agent consisting of 10-22% carbamide peroxide as the main ingredient is applied to a customized tray made to fit the patient's dentition, and the patient wears the tray and performs bleaching at home.

The following mechanism of bleaching has been reported by

Goldstein et al⁴. Hydroxyl radicals generated by decomposition of the main ingredient hydrogen peroxide or carbamide peroxide are unstable compounds possessing very strong polarity, and they cleave the color-imparting molecular chains of organic substances, converting them into small molecular substances with nearly no color. Therefore, bleaching is a process of decoloration by decomposing organic substances, and has been regarded to have no effect on inorganic substances^{5,6}. On the other hand, changes in micro structure of the enamel surface have been observed under a scanning electron microscope, and spot-like depressed structures have been reported. Reduced acid resistance has also been reported⁷⁻¹⁰.

There are few morphological studies on the changes of enamel resulting from bleaching^{11,12}. Especially, no study of the structural changes of crystals using ultra high resolution transmission electron microscope can be found in the literature. Therefore, the present study focused on the effects of two bleaching agents currently in clinical use on enamel crystals by observing the crystal morphology, and explored the difference in bleaching actions of the two agents and the resulting changes in crystal structures.

Materials and Methods

1. Experimental bleaching

Completely impacted third molars extracted from patients in their twenties were used, and the smooth enamel surface on the

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buccolingual side of the tooth was studied. Collection and use of the extracted teeth were approved by the Ethics Committee of Tokyo Dental College.

The enamel of extracted tooth was trimmed into a block (5 mm in length × 8 mm in width × 3 mm in height). The number of blocks was 50. The superficial layer was removed using No. 1000 waterproof abrasive and then polished with No. 4000 waterproof abrasive to expose fresh enamel. After ultrasonic cleaning, the tooth surface was divided into a control surface and a bleaching surface. The control surface was covered with dental wax. A window measuring 3 mm × 4 mm was made on the bleaching surface, at which bleaching was conducted using Hi-Lite (Hi-L: 35% hydrogen peroxide solution; Shofu, Japan) or Nite White Excel (NWE: 10% carbamide peroxide; Discus Dental Inc., USA). The number of blocks was 25 each. The main ingredients are shown in Table 1.

Bleaching was conducted in a moist chamber set at a temperature of 37°C and humidity at saturation point. The surfaces were treated with the maximum number of treatments for the maximum length of time described in the instructions of each product: 20 minutes × 18 sessions, a total of 6 hours for Hi-L; and 2 hours × 14 sessions, a total of 28 hours for NWE. After each bleaching, the surface was washed under running water, air dried, and then proceeded to the next bleaching. For Hi-L bleaching, light irradiation that accelerates the reaction to shorten chair time in the clinic was not used; instead bleaching was conducted in the thermostated box for 20 minutes that allows complete reaction, which was confirmed by noting a change of color of the indicator from blue to white.

After bleaching, the wax of the block was removed with xylene, and the specimens were observed by methods described below.

2. Observation Methods

1) Contact microradiography (CMR)

The specimen was dehydrated in graded ethanol and embedded in polyester resin (Rigolac, Nisshin-EM Co., Tokyo). Then ground sections 100 μm in thickness were prepared in a direction perpendicular to the surface. CMR was taken with a soft X-ray generator (CMR-3, Softex Co., Tokyo), and the degree of X-ray transmittance was evaluated. The photographic conditions were 10 kV, 3 mA, film-focus distance of 44.4 mm, and photographic time of 30 minutes. A high precision photo plate (HRP-SN-2,

Konica Minolta, Tokyo) was used as the photographic plate and the image was developed with D-19 developer (Kodak, USA) for 5 minutes.

2) Scanning Electron Microscope (SEM)

The specimen was dehydrated as described above. The ethanol was replaced by t-butyl alcohol and freeze-drying was conducted in a freeze dryer (ID-2, Eiko, Tokyo). The specimen was then sputter coated with Au-Pd, and the surface structure was examined under a field emission SEM (JSM-6340F, JEOL, Tokyo). The accelerating voltage was 15 kV.

3) Micro X-ray diffractometer (XRD)

The specimen was dehydrated and subjected to freeze drying as described for SEM. Qualitative analysis of the surface was conducted using a XRD (Rint 2500, Rigaku Electric, Tokyo). The conditions were 30 kV, 200 mA, collimator of 100 μm.

4) High-resolution transmission electron microscopy (HR-TEM)

The ground sections used in CMR observation were sandwich-embedded in epoxy resin (Epon 812), and ultrathin sections were prepared using a diamond knife. To observe the cross section of enamel prisms at the most superficial layer, ultrathin sections were prepared almost parallel to the surface. The unstained ultrathin sections were observed under a HR-TEM (EM-002B, Topcon, Tokyo) at an accelerating voltage of 200 kV.

Results

1) CMR observation

The surface of control enamel was flat and the degree of mineralization was homogeneous from the superficial layer down to deeper layers. On enamel bleached by both Hi-L and NWE, no remarkable changes were observed on the superficial layer of enamel (Figures 1-a,b,c). These results indicated that the bleaching agents caused almost no change in inorganic substances at the level of CMR.

2) SEM observation

The surface of control enamel was very smooth, and structures such as enamel prism were not visible (Figure 2-a). On the other hand, the surface bleached by Hi-L showed cross sections of enamel prisms. Narrow gaps were clearly observed in the prism sheath region of the head of prisms, while crack-like gaps were clearly visible on the whole surface (Figure 2-b). Similarly, the surface bleached by NWE also showed narrow gaps in the prism sheath region and cracks on the whole area, together with the appearance of granular material in some areas (Figure 2-c).

3) XRD observation

Qualitative analyses were conducted at 10 sites each of the control, Hi-L-bleached and NWE-bleached enamel surface. Comparing the sites with the highest confidence coefficients, calcium hydrogen phosphate hydroxide was detected in 8 sites and hydroxyapatite in 2 sites on the control enamel surface. On Hi-L-bleached enamel surface, calcium hydrogen phosphate hydroxide was detected at 5 sites and hydroxyapatite at 5 sites.

Table 1. Ingredients of the bleaching agents used in this study

<u>Hi Lite</u>
Liquid: 35% hydrogen peroxide solution, others
Powder: Aerosil, metallic salt, indicator, others
<u>Nite White Excel</u>
Main ingredient: 10% carbamide peroxide
Additives: vinyl polymer, propylene glycol, ethylene oxide, glycerol, cellulose, pH adjuster, fragrance

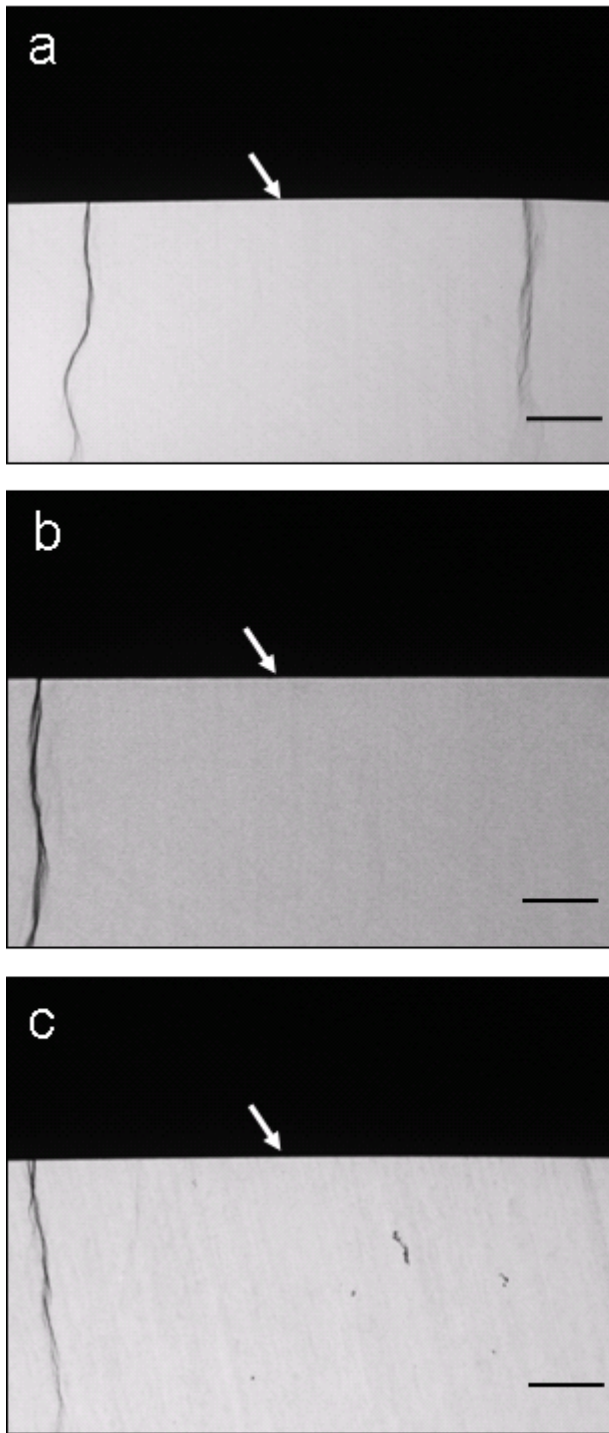


Figure 1. CMR of control enamel (a), Hi-L-bleached enamel (b) and NWE-bleached enamel (c). (Bars: 40 μ m)

No change in degree of mineralization is observed in three specimens. (Arrows: enamel surface)

On NWE-bleached enamel surface, calcium hydrogen phosphate hydroxide was found at 8 sites and hydroxyapatite at 2 sites (Table 2).

When the analytical charts were compared, all the peaks were sharp indicating high crystallinity at all enamel surfaces. These

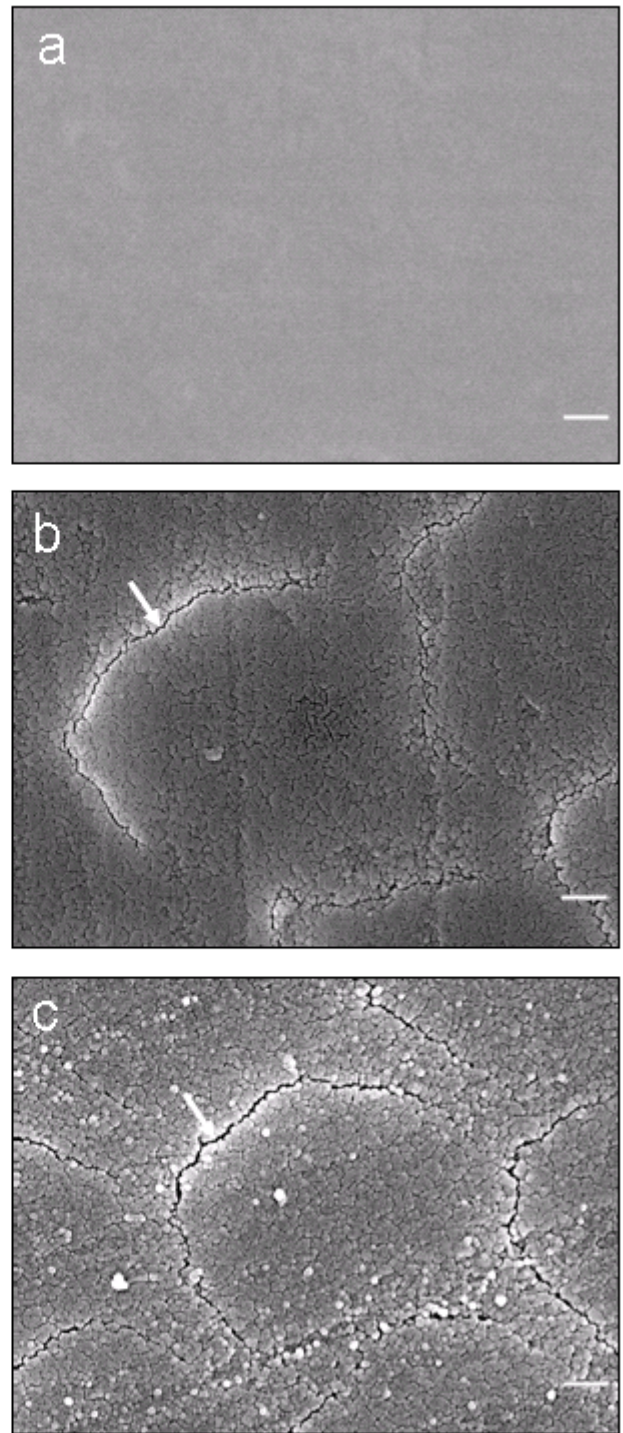


Figure 2. SEM of control enamel (a), Hi-L-bleached enamel (b) and NWE-bleached enamel (c). (Bars: 1 μ m)

Gaps along the prism sheath (arrows) and cracks between crystals are observed on the surface of Hi-L-bleached and NWE-bleached enamel specimens.

findings showed no changes in enamel crystallinity on control, Hi-L-bleached and NEW-bleached enamel surfaces, and that all had similar crystalline structures.

4) HR-TEM observation

At the superficial layer of control enamel, the c-axis cross

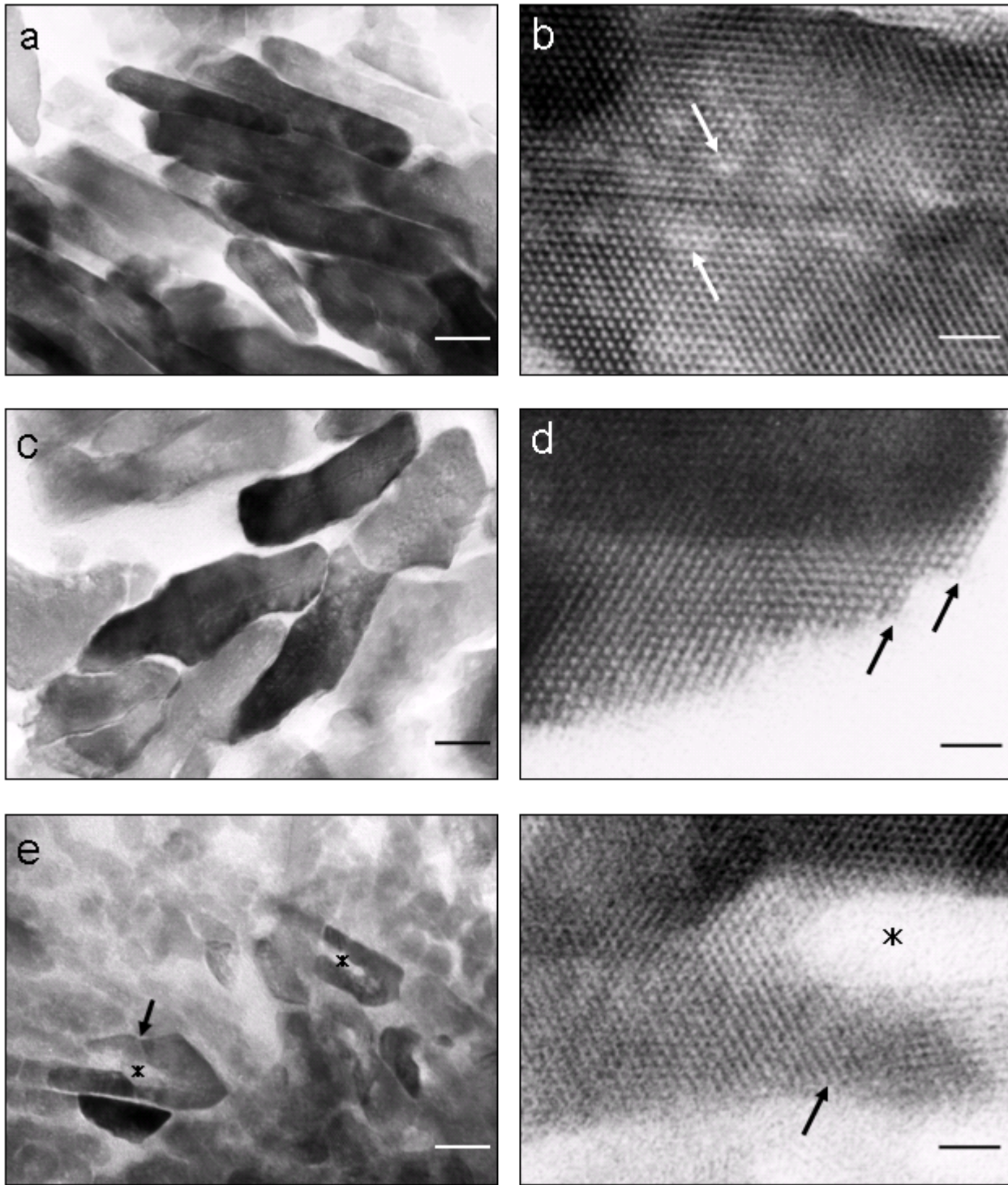


Figure 3. HR-TEM of control enamel (a, b), Hi-L-bleached enamel (c, d) and NEW-bleached enamel (e, f).

In control, (a) the *c*-axis cross section of enamel crystals shows an elongated flat hexagonal configuration, and (b) indefinite white spots (arrows) are observed at the surrounding area of central dark line. In Hi-L-, (c) the *c*-axis cross sections of the crystals are irregular, and (d) crystal growth toward the *a*-axis is observed (arrows). In NWE, (e) central perforation in the crystal (*) and dissolution from surrounding crystals (arrow) are observed, and (f) shows an enlarged image of dissolved crystal. (Bars: a,c,e, 30nm, b,d,f, 5nm)

section of enamel crystals showed an elongated flat hexagonal configuration. The crystal angles were sharp (Figure 3-a). Central dark lines were observed in the center of the crystals, with indefinite white spots in the surrounding area (Figure 3-b). Inside

the crystal, of crystal lattices intersecting at 60-degree angle in three directions were observed (Figure 3-b).

The crystals near the superficial layer of the Hi-L-bleached enamel showed a different configuration compared to the normal

Table 2

	Calcium hydrogen Phosphate hydroxide	Hydroxyapatite
Control	8	2
Hi-L	5	5
New	8	2

Comparing the sites with the highest confidence coefficients, calcium hydrogen phosphate hydroxide was detected in 8 sites and hydroxyapatite in 2 sites on the control and NEW samples. On Hi-L sample, calcium hydrogen phosphate hydroxide was detected at 5 sites and hydroxyapatite at 5 sites.

crystal structure observed on control enamel surface. The c-axis cross sections of the crystals were irregular. In some parts, crystal growth toward the a-axis were observed (Figures 3-c,d). On the one hand the crystal gaps were slightly wider than in control enamel, but the other hand became rather narrow in the regions showing marked crystal growth. Central dark lines were clearly found in the center of crystals, and white spots were decreased.

The crystals near the superficial layer of the NWE-bleached enamel appeared small and fragmented, some showing central perforation and dissolution from surrounding crystals was also observed (Figure 3-e,f). These crystals resembled the characteristic findings of caries crystals, showing clearly demineralization. The ordinary crystals seen in control enamel were almost absent, while crystal gaps were wider compared to control in most of the areas.

Discussion

Tooth whitening is achieved by a masking effect¹³⁾ achieved by producing micro changes such as roughness on the superficial layer of the enamel causing diffuse light reflection to cover up the internal color, and by a bleaching effect¹⁴⁾ achieved by decomposing the colored molecules with free radicals generated on the superficial layer of enamel.

According to Nakajima et al¹⁵⁾, the color change by tooth bleaching is limited to within 0.25 mm of the enamel superficial layer. On the other hand, Haywood et al³⁾, reported that hydrogen peroxide at the enamel superficial layer reaches the dentinoenamel junction, decomposing colored substances inside the dentine and removing the color.

A 35% hydrogen peroxide solution or 10% carbamide peroxide is usually employed in tooth bleaching. Carbamide peroxide is broken down into hydrogen peroxide and urea; the free radicals generated from hydrogen peroxide act on organic substances rendering pigmented large molecules into small molecular substances to achieve the bleaching effect. Therefore, the bleaching mechanisms do not differ greatly between the two agents. Furthermore, free radicals theoretically act on organic substances and have been thought to have no effect on inorganic substances⁵⁻⁶⁾. However, it has been reported that the superficial surface of enamel is rough after bleaching, losing the surface properties before bleaching⁷⁻¹⁰⁾. This suggests that demineralization may have oc-

curred because the pH is known to decrease when the bleaching agent acts¹³⁾. Kobayashi et al²⁾, reported that although the action is mild, repeated bleaching results in depressions around 2 μ m in diameter. However, no study has ever investigated the changes in crystal structure, and the detail remains unclear.

In the present study, when the effect of Hi-L was compared with NWE, no detectable effect of bleaching on dental enamel was detected by CMR. This finding probably reflects that the effect of bleaching on inorganic substances is very mild and limited to the most superficial layer.

SEM clearly depicted expansion of the prism sheath and narrow gaps resembling cracks between crystals for both bleaching agents. These gaps are consistent with the distribution pattern of organic substances in the enamel, and were probably formed by decomposition and removal of the organic substances. Inorganic substances occupy 89-91% by volume of enamel¹⁶⁾. Conversely, water and organic substances constitute only 10% by volume. Removal of these water and organic substances probably gives rise to narrow gaps in the prism sheath and between crystals.

By HR-TEM, opposite changes were observed in Hi-L-bleached enamel and NWE-bleached enamel. After bleaching with NWE, the most superficial layer showed clear findings of demineralization of enamel crystals, including central dissolution and crystal fragmentation as seen in caries crystals¹⁷⁾. The major ingredient of NWE is low concentration carbamide peroxide, and the duration of use is very long. As mentioned earlier, the carbamide peroxide breaks down into low concentration of hydrogen peroxide that acts on the dental surface in a sealed environment for a long period of time. While the pH change of NWE is unknown, pH is likely to be lowered when hydrogen peroxide acts. Despite the low concentration, a long duration of action (2 hours \times 14 sessions= 28 hours in this study) has a high possibility of inducing demineralization changes. Also from the viewpoint that NWE is used for home bleaching, caution is required when using this agent clinically.

Previous reports on the use of Hi-L have indicated that the pH falls to as low as 4.0 immediately after action and that the teeth are susceptible to demineralization by acid after bleaching, suggesting the possibility of demineralization¹³⁾. However, in the present study, demineralization findings were not evident; rather findings resembling crystal growth were observed. A possible explanation of the difference compared to NWE is the difference in duration of bleaching. The bleaching time with Hi-L (20 minutes \times 18 sessions= 6 hours) for each session was shorter than that with NWE. Therefore the low pH was probably effective to break down organic substances but had almost no effect on inorganic substances. Furthermore, the transient low pH is probably proportional to the amount of free radicals generated. Since light irradiation that accelerates the reaction was not used in this study, the extent of pH lowering was probably not sufficient for strong demineralization of crystals. In the present study, crystal growth

toward the direction of a-axis was observed. Usually, the enamel crystals are covered by enamel proteins¹⁶⁾. When the bleaching agent acts transiently and removes protein partially, the crystal surface is exposed. Deposition of apatite on the partially exposed crystal surface may give rise to partial crystal growth. In addition, observations of Hi-L-bleached enamel showed a reduction in white spots around the central dark lines and the highest rate of hydroxyapatite detection by XRD analysis. These findings support the notion that mineralization rather than demineralization is predominant in Hi-L-bleached enamel. The main sources of Ca and PO₄ ions utilized in mineralization are presumably decomposed organic substances and the ions released from brief demineralization from the exposed crystal surface, but the metallic salt contained in Hi-L may also play a role.

In this study, various effects of bleaching on dental enamel were observed. Especially, the changes in crystal structure observed using HR-TEM were in contrast to the conventional assumptions. Further study of this phenomenon may lead to the development of new tooth bleaching agent that provides bleaching effect with little damage to the dental enamel.

Conclusion

The effects of tooth bleaching on dental enamel were observed using CMR, SEM, XRD and HR-TEM.

1. On CMR, no change in degree of mineralization was observed in both Hi-L- and NWE-bleached enamel as compared with the control.
2. On SEM, gaps along the prism sheath and cracks between crystals were observed on the enamel surface in both Hi-L- and NWE-bleached enamel.
3. On XRD, no remarkable differences in crystal composition and crystallinity were observed as compared with the control.
4. On HR-TEM, crystal growth was observed in some parts of the Hi-L-bleached enamel, but crystal demineralization was observed in NWE-bleached enamel.
5. In all samples, bleaching only affected the most superficial layer of enamel.

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