

Title	Influence of peroxide treatment on bovine enamel surface : cross-sectional analysis
Author(s) Alternative	Ushigome, T; Takemoto, S; Hattori, M; Yoshinari, M; Kawada, E; Oda, Y
Journal	Dental materials journal, 28(3): 315-323
URL	http://hdl.handle.net/10130/1021
Right	

Influence of peroxide treatment on bovine enamel surface —Cross-sectional analysis—

Toshiaki USHIGOME, Shinji TAKEMOTO, Masayuki HATTORI, Masao YOSHINARI, Eiji KAWADA and Yutaka ODA

Department of Dental Materials Science, Tokyo Dental College, 1-2-2 Masago, Mihama-ku, Chiba 261-8502, Japan
Corresponding author, Shinji TAKEMOTO; E-mail: takemoto@tdc.ac.jp

Carbamide peroxide and hydrogen peroxide are used as the main agents in vital tooth bleaching. In this study, the influence of peroxide treatment on cross-sectional morphology and mechanical property was investigated. A 3×5-mm window of enamel on the labial surface of a bovine tooth was exposed to immersion in 10% or 30% carbamide peroxide or hydrogen peroxide for 30 or 180 min. After immersion, the cross-sectional structure of each specimen was examined by nanoindentation and SEM. Nanohardness in the enamel showed a decrease at 2 μm below the surface, but none at 50 μm. High concentrations of peroxide caused erosion to a depth of 5 μm below the surface. In conclusion, decrease in nanohardness and change in morphology were limited to an area less than 50 μm below the surface, regardless of either concentration of peroxide or period of immersion.

Keywords: Nanohardness, Peroxides, Cross-sectional structure

Received Sep 9, 2008; Accepted Jan 9, 2009

INTRODUCTION

Carbamide peroxide and hydrogen peroxide are often used in the bleaching of vital teeth to treat discoloration¹⁻⁷. High-concentration peroxides of 30% are used for in-office bleaching, and low concentrations of approximately 10% are used for at-home bleaching. However, the underlying mechanism of bleaching and potential side-effects such as hypersensitivity remain to be clarified.

Two bleaching mechanisms have been proposed: one suggests that peroxides cause slight morphologic alterations in the enamel which reduce its translucency by scattering light, so that the ensuing opaqueness masks the subjacent dentin layer⁸⁻¹¹ (frosted-glass effect); the other proposes that peroxide radicals, which are generated by the degradation of peroxide on the enamel surface, penetrate the enamel/dentin and break down the pigment of the discolored dentin^{12,13} (penetration effect). Since either of these mechanisms would involve dissolution of enamel and damage to the teeth, subsequent compromise of the mechanical properties of the teeth themselves is a matter of concern¹⁴.

Measuring tooth hardness is one way to evaluate change in mechanical properties. A number of studies using the Vickers and Knoop tests have reported a decrease in microhardness in enamel surfaces treated with peroxide solutions^{9,13,15,16}. However, the large load on, and large indentation in materials that these methods involve makes them unsuitable for measurement of hardness at the nano or micro level. Recently, a nanoindentation system capable of addressing this problem has drawn attention¹⁷⁻²⁰. With this method, only a small load is

required to induce an indent, and insertion depth is measured with a high-resolution displacement gauge to calculate hardness. This nanoindentation system offers a potential means of clarifying the effect of bleaching on the hardness of micro-regions in tooth.

In this study, to clarify the underlying mechanism of improvement of discolored teeth by peroxide, we treated bovine enamel with carbamide peroxide solution or hydrogen peroxide solution at different concentrations for 30 or 180 min. We investigated subsequent changes in enamel surface morphology, amount of dissolved mineral, and influence on cross-sectional morphology and nanohardness.

MATERIALS AND METHODS

Preparation of bovine tooth

Sixty-seven bovine teeth were prepared. Twenty-seven bovine teeth were used for surface morphology observation and roughness measurement, and another 40 bovine teeth were used for measurement of dissolved mineral, nanohardness measurement and cross-sectional morphology observation. After thawing cryopreserved bovine teeth at room temperature, the tooth crown, which was cut at the cemento-enamel junction, was used as a specimen. The specimens were polished with 1200-grid silicon carbide abrasive paper, ultrasonically washed in distilled water for 2 min to remove extraneous substances and coronal cementum from the labial enamel surface, and air-dried. The pulpal chamber was filled with resin (Unifast II, GC) to close the root canal. After attaching a piece of masking tape measuring 3×5 mm in size on the labial enamel

surface at a position 5 mm from the incisal edge, the enamel surface was covered with nail varnish. After drying, a masking tape was then removed, and a 3×5-mm window on the enamel surface was thus exposed before peroxide treatment.

Peroxide treatment

Carbamide peroxide solution and hydrogen peroxide solution (Hydrogen peroxide, Wako) were used at concentrations of 10% and 30%, respectively. The carbamide peroxide solution was composed of powder carbamide peroxide (Urea hydrogen peroxide, Sigma-aldrich) dissolved in distilled water.

Each specimen was placed in a bottle measuring 1 inch in diameter, into which 10 mL each peroxide solution was then poured. They were then placed in a thermostat bath at 30°C and left. The immersion times of each peroxide solution were 30 or 180 min. Type of peroxide, concentration and pH of solution, and code of each specimen are shown in Table 1. After immersion, the specimens were removed from the bottle, washed in distilled water, and air-dried.

Surface morphology observation and roughness measurement

The peroxide-treated specimens were dried at room temperature more than 24 hours to avoid enamel crack. The specimen surface was gold sputter-coated and observed under field emission scanning electron microscopy equipped with electron beam 3D surface roughness analyzer (SEM; ERA-8900FE, Elionix). The peroxide-treated area measuring 90×120 μm was then analyzed under an accelerating voltage of 15 kV to determine surface roughness (*Sa*) by electron beam 3D surface roughness analyzer. As a control specimen, the enamel surfaces were only polished, that is, they not treated with any peroxide solutions. Three specimens were measured under each condition.

Measurement of dissolved mineral

Amount of calcium and phosphorus in the solution after immersion was determined using inductively-coupled plasma atomic emission spectroscopy (ICP: Vista-MPX, SII), and 5 specimens were subjected to each condition.

Nanohardness measurement

After immersion, specimens were fixed in a 1-inch epoxy ring perpendicular to the tooth axis and embedded in a self-curing epoxy resin (Scandiplex, Scandia). After the resin was cured, the embedded specimen was cut at an angle perpendicular to the tooth axis 7 mm from the incisal edge of the bovine tooth. Next, the cross-sectional specimen was mirror-polished with 320-grid to 1200-grid silicon carbide abrasive paper using an automatic polishing machine (Automet2 & Ecomet3, Buehler), and then polished again with a 0.05-μm alumina suspension to section the specimen. The polished specimen was then ultrasonically washed in distilled water for 2 min.

The nanohardness of the enamel section was then determined using a nanoindentation system (ENT-1100a, Elionix). The load was 200 mgf, loading and unloading speeds were 0.02 mgf/ms, and retention time was 1000 ms. Measurements were performed on the peroxide-treated and nail varnish-covered areas in each specimen (denoted as H_{PO} and H_{NV} , respectively). The nail varnish-covered areas consisted of those sections of the enamel surface that did not come into contact with the peroxide solutions. Nanohardness was measured from the outermost surface of the enamel at intervals of 2 μm within an area from 2 to 20 μm below the enamel surface, and then at 50, 100, 200, and 400 μm below the enamel surface. The measurements were made at 3 points within each region, and the mean value was calculated as the hardness at that region. Five specimens were measured under each condition. Differences in nanohardness (ΔH) between peroxide-treated areas and nail varnish-covered areas on each tooth were calculated ($\Delta H: H_{PO} - H_{NV}$) at each region from the outermost enamel.

Cross-sectional morphology observation

SEM observations of the sectional specimen after measuring nanohardness were performed at the peroxide-treated and nail varnish-covered areas.

Statistical analysis

Surface roughness (*Sa*), concentration of dissolved elements in the solution, and cross-sectional nanohardness were statistically analyzed using a one way analysis of variance (ANOVA) and Scheffe's multiple comparison test at a significance level of 95%.

Table 1 Type of peroxide, concentration and pH of solution, and code of specimen

Solution	Concentration (mass%)	pH	Code
Carbamide peroxide	10	4.6	10CP
	30	4.3	30CP
Hydrogen peroxide	10	4.7	10HP
	30	3.6	30HP

RESULTS

Surface morphology

Fig. 1 shows SEM photographs of representative enamel surfaces on specimen with or without peroxide treatments. Only a polished scratch was observed on the specimens without peroxide treatment (Fig. 1(a)). In Figs. 1(b) and (c), the 30CP specimen immersed for 30 min showed a roughened surface, whereas the 10HP specimen immersed for 30 min showed only a polished scratch, as in Fig. 1(a). With 180 min immersion, the 30CP and 10HP specimens showed rougher surfaces than those immersed for 30 min (Figs. 1(d) and (e)). In addition, the 10HP specimen showed a groove that appeared to be an eroded enamel rod sheath. Although not shown in the figure, the 10CP specimen showed the same morphology as the nail-varnish covered area with 30 min immersion, and the surface was smoother than that obtained with 180 min immersion. The 30HP specimen revealed a groove that appeared to be an eroded enamel rod sheath with 30 and 180 min immersion, as shown in Fig. 1(e).

Surface roughness

The surface roughness (*Sa* value) of specimen without peroxide treatment was $0.034 \pm 0.008 \mu\text{m}$. Fig. 2 shows the *Sa* value of enamels immersed in each peroxide solution for 30 and 180 min. The *Sa* values of the 10CP specimen with 30 and 180 min

immersion were 0.039 ± 0.007 and $0.062 \pm 0.013 \mu\text{m}$, respectively. The 10CP specimen showed a larger *Sa* value with 180 min immersion than with 30 min immersion ($p < 0.05$). Similarly, the *Sa* values of the 10HP and 30HP specimens with 30 min immersion were 0.040 ± 0.004 and $0.044 \pm 0.007 \mu\text{m}$, respectively, values smaller than 0.069 ± 0.015 and $0.079 \pm 0.023 \mu\text{m}$, which were the values with 180 min immersion ($p < 0.05$).

With 30 min immersion, the *Sa* value of the 30CP specimen was larger than those for the 10CP and 10HP specimens ($p < 0.05$), whereas no significant difference was observed between the *Sa* values of the specimens with 180 min immersion.

Amount of released mineral

Amounts of dissolved phosphorus could not be compared, as a lot of phosphorus was detected in the prepared carbamide peroxide solution. Concentration of calcium in the solution before immersion of the 10CP, 30CP, 10HP, and 30HP specimens was 0.03 ± 0.01 , 0.08 ± 0.03 , 0.01 ± 0.00 , and 0.01 ± 0.01 ppm, respectively. Fig. 3 shows the amount of calcium dissolved from each specimen with 30 min and 180 min immersion. The amount of calcium dissolved from the 10CP specimen with 30 min and 180 min immersion was 12 ± 1 and $41 \pm 10 \mu\text{g}/\text{cm}^2$, respectively, with greater dissolution occurring with 180 min immersion than with 30 min ($p < 0.05$). Furthermore, the 30CP, 10HP, and 30HP specimens showed 24 ± 5 , 8 ± 1 , and $14 \pm 2 \mu\text{g}/\text{cm}^2$ dissolved calcium, respectively,

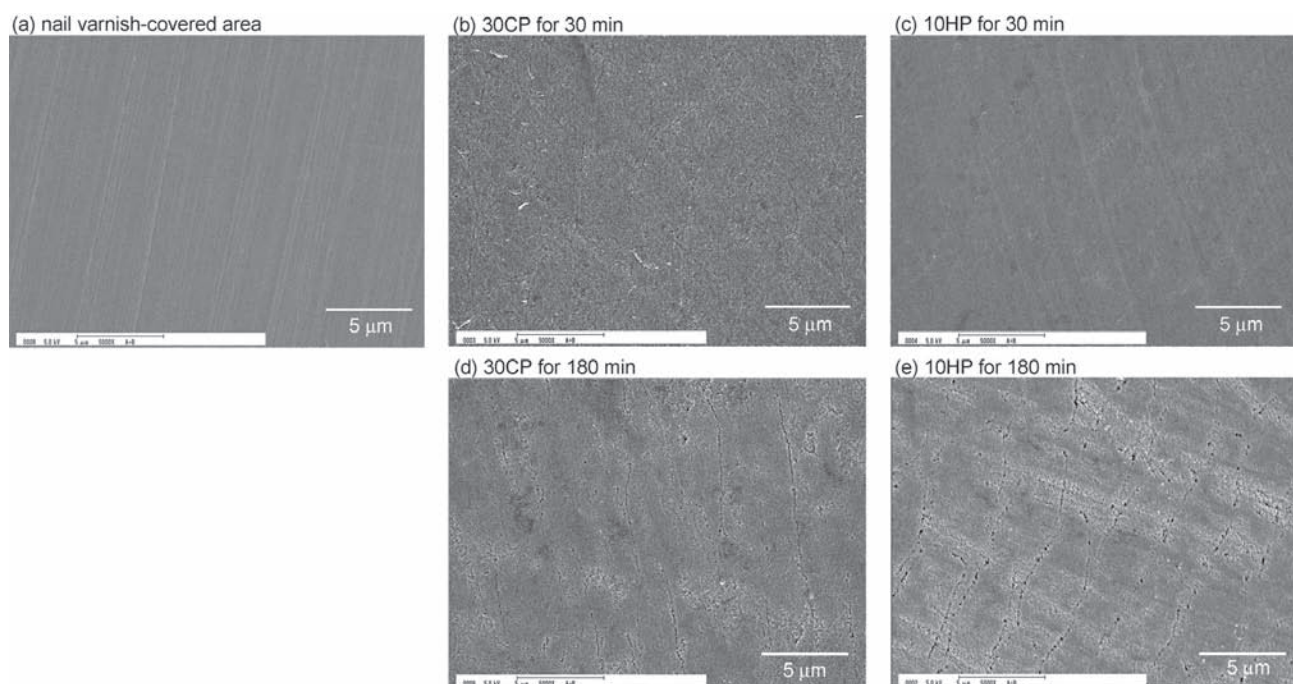


Fig. 1 SEM photographs of enamel surface of nail varnish-covered area (a) and peroxide-treated area (b-e) in teeth immersed in peroxide solutions. (a) nail varnish-covered area, (b) 30CP for 30 min, (c) 10HP for 30 min, (d) 30CP for 180 min, (e) 10HP for 180 min

with 30 min immersion, and 60 ± 11 , 19 ± 5 , and $46 \pm 4 \mu\text{g}/\text{cm}^2$ dissolved calcium, respectively, with 180 min immersion. Amount of dissolution in all specimens was similar in that it increased with period of immersion ($p < 0.05$). The amount of calcium dissolved from the 30CP specimen was larger than that from the 10HP specimen ($p < 0.05$).

Change in nanohardness of cross-sectional enamel

Fig. 4 shows the typical nanohardness (H_{PO} and H_{NV}) of the peroxide-treated and nail varnish-covered areas on a 30CP specimen with 180 min immersion. The H_{NV} indicated about 7–8 GPa at 2 μm from the outermost surface to a depth of 400 μm . On the other hand, the H_{PO} was 4 GPa at 2 μm from the outermost surface, showing a smaller value than that

for H_{NV} at the same distance. H_{PO} increased with increase in depth, approaching the same value as that for H_{NV} .

Fig. 5 shows difference in hardness (ΔH) of the cross-section at 2, 20, and 50 μm below the outermost surface of the enamel with 30 min immersion (a) and

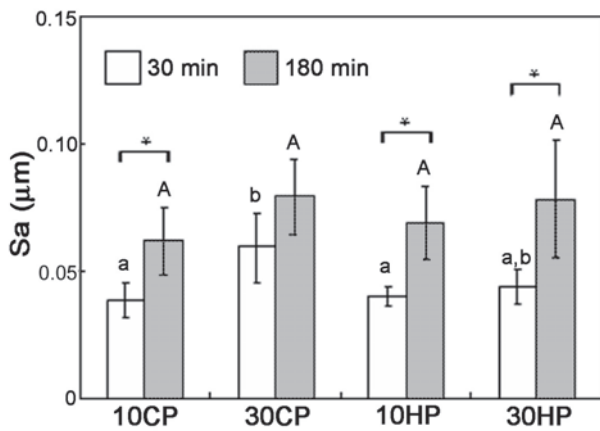


Fig. 2 Sa values of peroxide-treated areas on each enamel surface immersed in peroxide solution. Sa values of control area was $0.034 \pm 0.008 \mu\text{m}$. Asterisk indicates significant difference ($p < 0.05$). Groups with same letter showed no significant difference ($p > 0.05$).

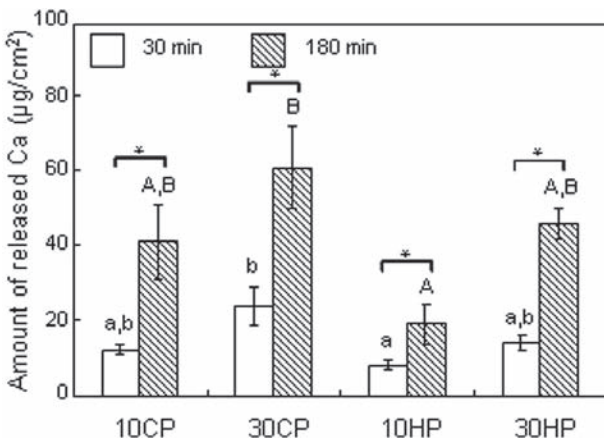


Fig. 3 Amount of calcium dissolved from enamel immersed in each peroxide solution. Groups with the same letter showed no significant difference ($p > 0.05$).

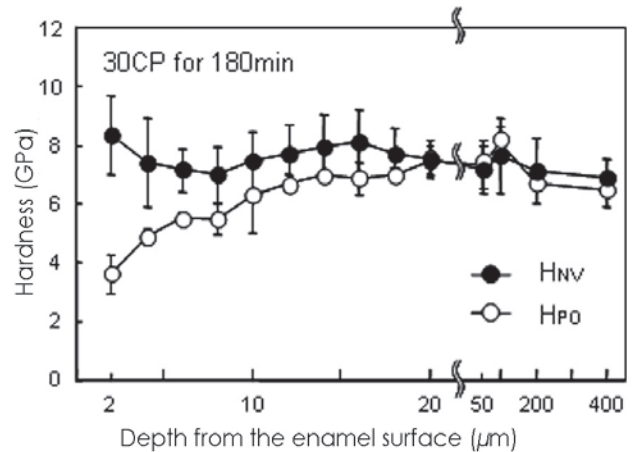


Fig. 4 Typical nanohardness of cross-sectional 30CP specimen with 180 min immersion. Open circle indicates depth profile of H_{PO} (peroxide-treated areas); closed circle indicates that of H_{NV} (nail varnish-covered areas).

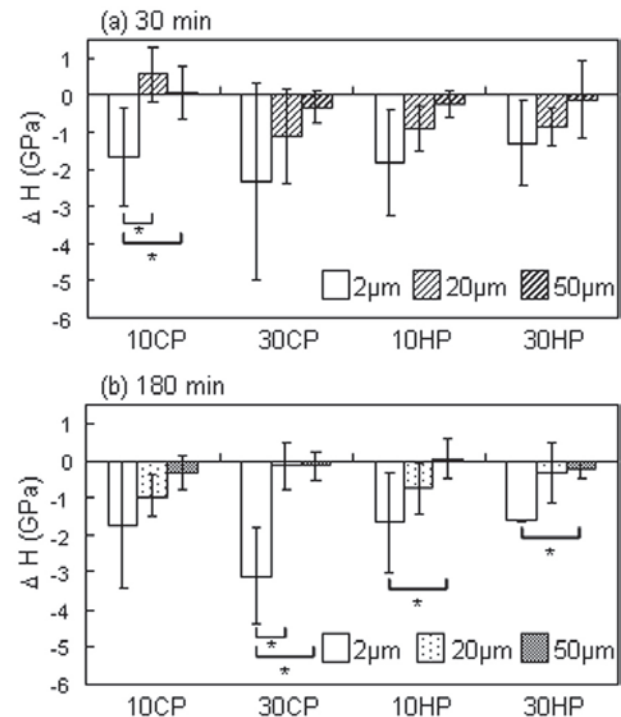


Fig. 5 Difference in nanohardness (ΔH) at 2, 20, and 50 μm below enamel surface with immersion in peroxide solution. Asterisk indicates significant difference ($p < 0.05$). (a) 30 min immersion, (b) 180 min immersion.

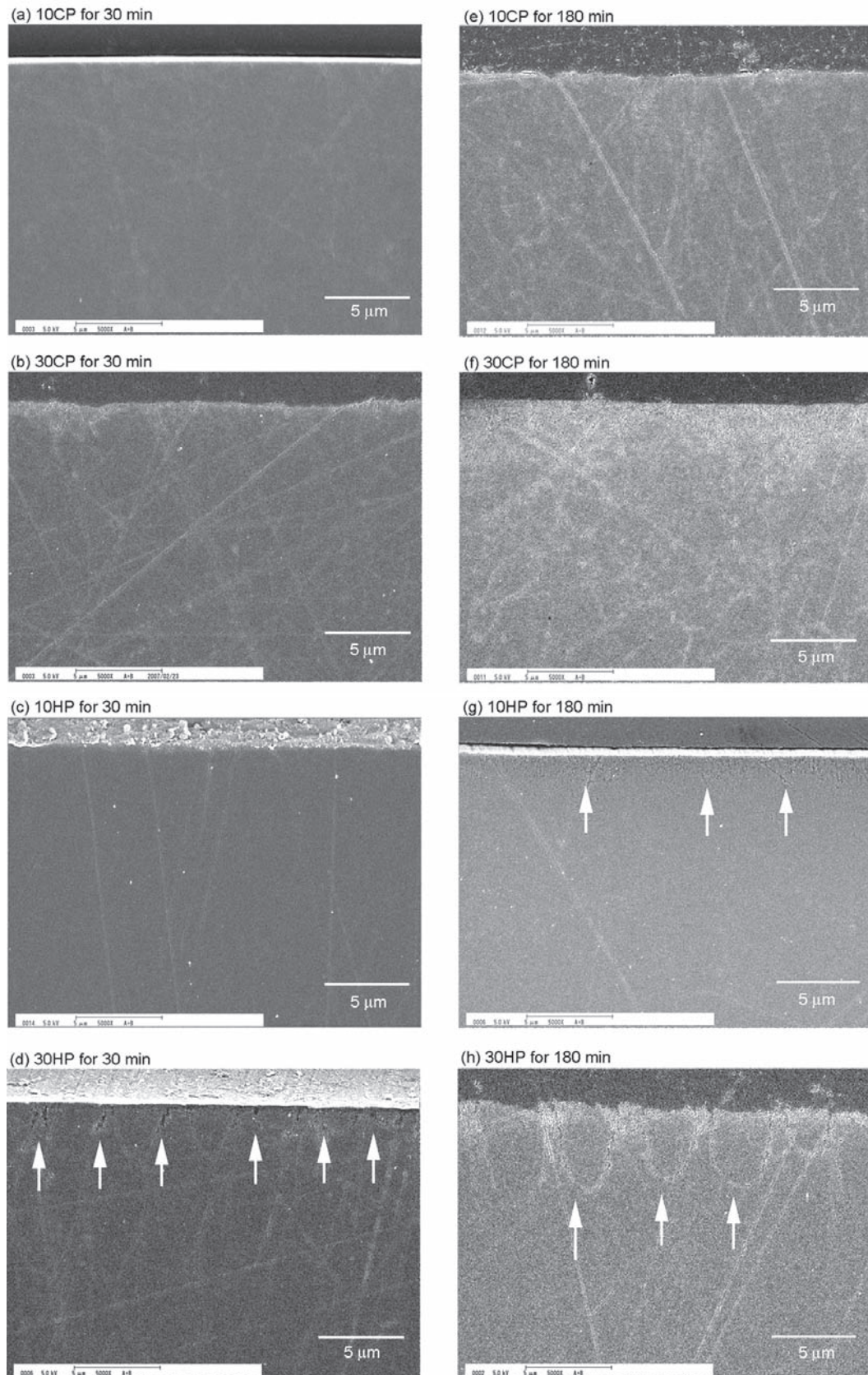


Fig. 6 SEM photographs of enamel immersed in peroxide solution for 30 and 180 min. Arrows indicate enamel rod sheaths. (a) 10CP for 30 min, (b) 30CP for 30 min, (c) 10HP for 30 min, (d) 30HP for 30 min, (e) 10CP for 180 min, (f) 30CP for 180 min, (g) 10HP for 180 min, (h) 30HP for 180 min.

180 min immersion (b). As seen in Fig. 5(a), the ΔH_s at 2, 20, and 50 μm in the 10CP were -1.67 ± 1.31 , 0.55 ± 0.74 , and 0.06 ± 0.70 GPa, respectively, showing the smallest value at 2 μm ($p < 0.05$). No significant difference was observed in the ΔH_s at 2, 20, and 50 μm in the 30CP, 10HP, and 30HP specimens. As shown in Fig. 5(b), 10CP specimen indicated no significant difference among ΔH_s at 2, 20, and 50 μm . The ΔH_s at 2, 20, and 50 μm in 30CP specimen were -3.10 ± 1.30 , -0.12 ± 0.65 , and -0.14 ± 0.39 GPa, respectively, with the smallest value for a depth of 2 μm ($p < 0.05$). The ΔH_s at 2 and 50 μm were -1.65 ± 1.34 and 0.05 ± 0.56 GPa, respectively, for 10HP specimen, and -1.59 ± 0.04 and -0.23 ± 0.23 GPa, respectively, for 30HP specimen, revealing the smallest value for a depth of 2 μm ($p < 0.05$).

Cross-sectional morphology

Fig. 6 shows SEM photographs of cross-sectional enamel immersed in each peroxide solution for 30 and 180 min. The outermost surface of the 30CP and 30HP specimens, which had come into contact with the peroxide solutions, became rough with 30 min immersion (Figs. 6(b) and (d)), whereas no change was observed in the 10CP or 10HP specimens with the same period of immersion (Figs. 6(a) and (c)). A groove that appeared to be an eroded enamel rod sheath was observed in the 30HP specimen with 30 min immersion (Fig. 6(d)).

All specimens showed a rough surface with 180 min immersion in either solution. The rough enamel regions of the 30CP and 30HP specimens with 180 min immersion were 5 μm below the outermost surface (Figs. 6(f) and (h)), and were deeper than those in the 10CP and 10HP specimens with 180 min immersion (Figs. 6(e) and (g)). With 180 min immersion, a groove that appeared to be an eroded enamel rod sheath was observed in enamel immersed in hydrogen peroxide. The groove in the 30HP specimen was more clearly visible than that in the 10HP specimen (Figs. 6(g) and (h)).

DISCUSSION

In-office bleaching uses bleaching agents that employ high concentrations of carbamide peroxide or hydrogen peroxide, and treatment time conforms with the manufacturer's recommendations²¹. This means that, on any given day, peroxide treatment is usually performed for 3 sets of 10 min each, to give a total treatment time of 30 min. This process is then repeated on further days, to give a total of 180 min (6 days \times 30 min). In-office bleaching may compromise the mechanical properties of the tooth due to the high concentration of peroxide used. Therefore, at-home bleaching using low concentrations of peroxide is also practiced. Taking

this into consideration, in this study, we immersed bovine enamel in low (10%) and high (30%) concentrations of carbamide peroxide or hydrogen peroxide for 30 or 180 min, and investigated surface and cross-sectional morphology, roughness, amount of dissolved mineral, and hardness.

Surface morphology and roughness

When enamel is treated with solutions with a lower pH than the critical pH of enamel (pH 5.5), the enamel may dissolve due to acidity^{22,23}. Some reports have found that calcium was dissolved from human enamel treated with commercial bleaching agents with a pH of 4.7–5.3 containing 10% carbamide peroxide^{24,25}. In addition, in enamel treated with hydrogen peroxide, a groove was observed which appeared to be an eroded enamel rod sheath, suggesting that peroxide affects the organic constituents of enamel^{21,26,27}. Decalcification has been reported in enamel treated with carbamide peroxide with a pH of 6.7–6.8, which is higher than the critical pH^{28,29}. Decalcification and morphological change in the enamel resulted from the increased surface roughness of the enamel brought about by peroxide treatment⁸⁻¹¹. As mentioned above, when treating enamel with bleaching agents containing peroxides, dissolution will occur depending on the pH of the solution and the type of peroxide used. Consequently, the enamel surface may become rough.

Since the pH of the solutions used in this study was lower than the critical pH of enamel (shown in Table 1), the dissolution of calcium from the teeth was easily explained. It should be noted that the amount of dissolved calcium was larger for carbamide peroxide than for hydrogen peroxide, and that this value increased with increase in concentration. On the other hand, surface roughness increased with increase in amount of dissolved calcium with 30 min treatment. These results suggest that surface roughness is associated with dissolution of tooth constituents.

No significant difference in terms of surface roughness of enamel was observed among type or concentration of peroxide with 180 min immersion. However, cross-sectional morphology revealed erosion of enamel with immersion (Fig. 6), depending on type of peroxide. This erosion was widespread, extending down to approximately 5 μm below the outermost surface with 180 min immersion. Erosion in the carbamide peroxide-treated enamel was uniform and at a constant distance from the outermost surface, whereas erosion in the hydrogen peroxide-treated enamel was selective and located in an enamel rod sheath-like area. Thus, the pattern of erosion differed between carbamide peroxide and hydrogen peroxide. In this study, we confirmed that carbamide peroxide contained phosphorus. Phosphorus may

become phosphoric acid in such solutions, thus resulting in etching of the enamel. Although carbamide peroxide may contribute to bleaching by decomposing into hydrogen peroxide and urea, other additives in the peroxide should be also taken into consideration.

Hardness of cross-sectional enamel

A number of reports have used the Vickers and Knoop tests or nanoindentation to investigate hardness in teeth treated with bleaching agents^{9,13,15,16,21,30-32}. However, these tests place an indentation load of 100–200 gf on the specimen and, therefore, require a width of approximately 20–40 μm for measurements to be made. This renders these tests unsuitable for measurement of changes in hardness at the nano or micro level^{30,31}.

The nanoindentation system used in this study can measure hardness with a load of 200 mgf and an indentation of less than approximately 1 μm , thus allowing evaluation of changes in the supersurface at 2 μm below the enamel surface. A number of factors may affect the hardness of enamel, including differences in individual teeth and the orientation of enamel rods. Therefore, the hardness of a peroxide-treated section and a nail varnish-covered section in each tooth were measured perpendicularly to the tooth axis and at a constant distant from the incisal edge, and comparisons were performed based on differences in hardness (ΔH).

The ΔH value at 2 μm below the enamel surface was found to be negative with immersion in the peroxide solutions. This indicated a decrease in nanohardness on the outermost surface of the peroxide-treated enamel. Furthermore, this decrease did not depend on type or concentration of peroxide, or period of immersion. The ΔH values at 50 μm below the enamel surface were all close to zero, indicating that the nanohardness of the nail varnish-covered section and peroxide-treated section were almost equal. In a study on bovine enamel treated with a commercial bleaching agent according to the manufacturer's instructions, Sekine et al found that nanohardness showed a decrease at up to 50 μm below the enamel surface²¹. Their study also investigated effect of type of bleaching agent, additives in bleaching agent, and light irradiation on acceleration of bleaching, which may explain why they found a decrease in hardness at a deeper level than that observed in this study. However, even if such accelerated bleaching were used clinically, decrease in hardness would be limited to a depth of approximately 50 μm below the outermost surface.

Mechanism for bleaching of discolored teeth

Cross-sectional morphological observation revealed that carbamide peroxide induced widespread erosion,

whereas hydrogen peroxide induced dissolution limited to an area which appeared to be made up of enamel rod sheaths. Although peroxide at a 30% concentration elicited deeper erosion, this was still only 5 μm below the outermost surface, even with 180 min immersion. On the other hand, a decrease in nanohardness was observed, regardless of type or concentration of peroxide. Hardness at a depth of 50 μm showed no decrease, regardless of which peroxide solution was used.

The underlying mechanism of bleaching of discolored teeth has been suggested to involve either a frosted-glass effect^{8,11} or a penetration effect^{12,13}. A cause of bleaching may be the scattering of light through roughening of the enamel surface; that is to say, the translucence of enamel may decrease with increase in surface roughness. On the other hand, the possible decomposition of pigment into dentin due to penetration of peroxide radicals cannot be ruled out³³. In this study, it is considered that peroxides may have penetrated deeper through microcracks or defects in the enamel, as well as through grooves that appeared to be eroded enamel rod sheaths, in enamel treated with hydrogen peroxide. This supports the finding of an early study which suggested that penetration by radicals contributes to bleaching by decomposition of pigment³⁴.

CONCLUSIONS

We investigated the influence of type and concentration of peroxide and immersion time on tooth structure surface morphology and nanohardness to clarify the mechanism by which peroxide bleaches discolored teeth. The results may be summarized as follows:

1. With immersion in carbamide peroxide or hydrogen peroxide solution, the surface roughness of bovine enamel increased due to dissolution of enamel constituents; erosion increased with increase in immersion time.
2. Regardless of type or concentration of peroxide, or immersion time, a partial decrease in nanohardness was observed at 20 μm below the outermost surface of the enamel, but no decrease in nanohardness was observed at 50 μm .
3. Carbamide peroxide elicited complete erosion, whereas hydrogen peroxide induced only partial erosion limited to an area that appeared to be made up of enamel rod sheaths. Higher concentrations of peroxides affected the enamel to a greater depth, although this only extended 5 μm below the outermost surface, even with 180 min immersion in either solution.

The results indicate that, while contact with peroxide induced erosion, decrease in nanohardness

and change in morphology were limited to a depth of less than 50 μm below the outermost surface.

ACKNOWLEDGMENTS

This study was supported in part by a Grant-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan. We would also like to thank Associate Prof. Williams, J. of Tokyo Dental College, for his assistance with the English of this manuscript.

REFERENCES

- 1) Goo D, Kwon T, Nam S, Kim H, Kim K, Kim Y. The efficiency of 10% carbamide peroxide gel on dental enamel. *Dent Mater J* 2004; 23: 522-527.
- 2) Matis BA, Cochran MA, Franco M, Al-Amr W, Eckert GJ, Stropes M. Eight in-office tooth whitening systems evaluated in vivo: a pilot study. *Oper Dent* 2007; 32: 322-327.
- 3) Gallagher A, Maggio B, Bowman J, Borden L, Mason S, Felix H. Clinical study to compare two in-office (chairside) whitening systems. *J Clin Dent* 2002; 13: 219-224.
- 4) Kugel G, Petkevis J, Gurgan S, Doherty E. Separate whitening effects on enamel and dentin after fourteen days. *J Endod* 2007; 33: 34-37.
- 5) Goodson JM, Tavares M, Sweeney M, Stultz J, Newman M, Smith V, Regan EO, Kent R. Tooth whitening: tooth color changes following treatment by peroxide and light. *J Clin Dent* 2005; 16: 78-82.
- 6) Duschner H, Gotz H, White DJ, Kozak KM, Zoladz JR. Effects of hydrogen peroxide bleaching strips on tooth surface color, surface microhardness, surface and subsurface ultrastructure, and microchemical (Raman spectroscopic) composition. *J Clin Dent* 2006; 17: 72-78.
- 7) Gotz H, Duschner H, White DJ, Klukowska MA. Effects of elevated hydrogen peroxide 'strip' bleaching on surface and subsurface enamel including subsurface histomorphology, micro-chemical composition and fluorescence changes. *J Dent* 2007; 35: 457-466.
- 8) Markovic L, Jordan RA, Lakota N, Gaengler P. Micromorphology of enamel surface after vital tooth bleaching. *J Endod* 2007; 33: 607-610.
- 9) Pinto CF, Oliveira R, Cavalli V, Giannini M. Peroxide bleaching agent effects on enamel surface microhardness, roughness and morphology. *Braz Oral Res* 2004; 18: 306-311.
- 10) Cavalli V, Arrais CA, Giannini M, Ambrosano GM. High-concentrated carbamide peroxide bleaching agents effects on enamel surface. *J Oral Rehabil* 2004; 31: 155-159.
- 11) Fu B, Hoth-Hannig W, Hannig M. Effects of dental bleaching on micro- and nano-morphological alterations of the enamel surface. *Am J Dent* 2007; 20: 35-40.
- 12) Gokay O, Tuncbilek M, Ertan R. Penetration of the pulp chamber by carbamide peroxide bleaching agents on teeth restored with a composite resin. *J Oral Rehabil* 2000; 27: 428-431.
- 13) Faraoni-Romano JJ, Turssi CP, Serra MC. Concentration-dependent effect of bleaching agents on microhardness and roughness of enamel and dentin. *Am J Dent* 2007; 20: 31-34.
- 14) Yazaki K, Kawada E, Oda Y. An evaluation of the penetration of peroxide from tooth-whitener. *Biomed Res* 2003; 24: 325-330.
- 15) Park HJ, Kwon TY, Nam SH, Kim HJ, Kim KH, Kim YJ. Changes in bovine enamel after treatment with a 30% hydrogen peroxide bleaching agent. *Dent Mater J* 2004; 23: 517-521.
- 16) Basting RT, Rodrigues Junior AL, Serra MC. The effect of 10% carbamide peroxide bleaching material on microhardness of sound and demineralized enamel and dentin in situ. *Oper Dent* 2001; 26: 531-539.
- 17) Habelitz S, Marshall GW Jr, Balooch M, Marshall SJ. Nanoindentation and storage of teeth. *J Biomech* 2002; 35: 995-998.
- 18) Cuy JL, Mann AB, Livi KJ, Teaford MF, Weihs TP. Nanoindentation mapping of the mechanical properties of human molar tooth enamel. *Arch Oral Biol* 2002; 47: 281-291.
- 19) Chng HK, Ramli HN, Yap AU, Lim CT. Effect of hydrogen peroxide on intertubular dentine. *J Dent* 2005; 33: 363-369.
- 20) Habelitz S, Marshall SJ, Marshall GW Jr, Balooch M. Mechanical properties of human dental enamel on the nanometre scale. *Arch Oral Biol* 2001; 46: 173-183.
- 21) Sekine G, Hotta M, Yamamoto K, Sekine I. Alterations in bovine enamel surface properties by bleaching with Hi-lite. *Jpn. J. Conserv. Dent* 2006; 49: 458-467.
- 22) Schmidt-Nielsen B. The solubility of tooth substance in relation to the composition of saliva. *Acta odont. scand.* 1946; 7 Suppl 2: 1-86.
- 23) Driessens FC, Theuns HM, Borggreven JM, van Dijk JW. Solubility behaviour of whole human enamel. *Caries Res* 1986; 20: 103-110.
- 24) McCracken MS, Haywood VB. Demineralization effects of 10 percent carbamide peroxide. *J Dent* 1996; 24: 395-398.
- 25) Lee KH, Kim HI, Kim KH, Kwon YH. Mineral loss from bovine enamel by a 30% hydrogen peroxide solution. *J Oral Rehabil* 2006; 33: 229-233.
- 26) Hegedus C, Bistey T, Flora-Nagy E, Keszthelyi G, Jenei A. An atomic force microscopy study on the effect of bleaching agents on enamel surface. *J Dent* 1999; 27: 509-515.
- 27) Shellis RP. A scanning electron-microscopic study of solubility variations in human enamel and dentine. *Archs oral Biol* 1996; 41: 473-484.
- 28) Efeoglu N, Wood DJ, Efeoglu C. Thirty-five percent carbamide peroxide application causes in vitro demineralization of enamel. 2007; 23: 900-904.
- 29) Efeoglu N, Wood D, Efeoglu C. Microcomputerised tomography evaluation of 10% carbamide peroxide applied to enamel. *J Dent* 2005; 33: 561-567.
- 30) Attin T, Vollmer D, Wiegand A, Attin R, Betke H. Subsurface microhardness of enamel and dentin after different external bleaching procedures. *Am J Dent* 2005; 18: 8-12.

-
- 31) Potocnik I, Kosec L, Gaspersic D. Effect of 10% carbamide peroxide bleaching gel on enamel microhardness, microstructure, and mineral content. *J Endod* 2000; 26: 203-206.
- 32) Hairul Nizam BR, Lim CT, Chng HK, Yap AU. Nanoindentation study of human premolars subjected to bleaching agent. *J Biomech* 2005; 38: 2204-2211.
- 33) Jiang T, Ma X, Wang Y, Zhu Z, Tong H, Hu J. Effects of hydrogen peroxide on human dentin structure. *J Dent Res* 2007; 86: 1040-1045.
- 34) Kawamoto K, Tsujimoto Y. Effects of the hydroxyl radical and hydrogen peroxide on tooth bleaching. *J Endod* 2004; 30: 45-50.