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치의학박사학위논문

**The effect of  
bleaching and remineralizing agent  
on color and chemical / mechanical  
properties of artificial initial carious lesions**

미백제 및 재광화제 사용이  
인공 초기 우식 병소의  
색 및 기계화학적 성질에 미치는 영향

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김 연 주

**The effect of  
bleaching and remineralizing agent  
on color, chemical / mechanical properties  
of artificial initial carious lesions**

**YOUNJU KIM, D.D.S.**

Department of Dentistry, Graduate School, Seoul National  
University

(Directed by Professor Ho-Hyun Son, D.D.S., M.S.D., Ph D.)

**Objectives**

The purpose of this study was to evaluate the effect of bleaching and remineralization with casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) on color and chemical / mechanical properties of teeth with white spot lesions.

## Materials & Methods

Carious lesions with standardized whiteness were produced on the buccal and lingual surfaces of human premolars by pH cycling. Specimens were subjected to four experimental conditions ( $n = 20/\text{group}$ ) as follows: Group 1, control; Group 2, caries formation followed by remineralization using fluoride-containing CPP-ACP (Tooth Mousse Plus, GC); Group 3, caries formation followed by bleaching using 10% carbamide peroxide; Group 4, caries formation followed by both bleaching and remineralization. The CIEL<sup>\*</sup>*a*<sup>\*</sup>*b*<sup>\*</sup> values were measured with a spectroradiometer, the mineral content was measured with electron probe microanalysis (EPMA) on the cross-sectional surface of each specimen, and the Knoop microhardness test was carried out along the EPMA scan line. Two-way analysis of variance was performed with Tukey *post hoc* comparison.

## Results

The CIEL<sup>\*</sup>*a*<sup>\*</sup>*b*<sup>\*</sup> values of the specimens at baseline (BA) and after caries formation (CA) were not significantly different among all groups. The change in the CIEL<sup>\*</sup>*a*<sup>\*</sup>*b*<sup>\*</sup> values was not significantly different between the caries-formed ( $\Delta E^* = 7.03$ ) and the bleached enamel ( $\Delta E^* = 7.60$ ). Bleaching of the carious enamel extended the whiteness ( $\Delta E^* = 3.38$ ) ( $p < 0.05$ ) without additional mineral loss. The remineralization treatment significantly increased the calcium (Ca), phosphate (P), and fluoride content of the subsurface lesion area ( $p < 0.05$ ). Each

microhardness value of the surface, subsurface, and total lesion area did not show a significant difference across groups. The cross-sectional microhardness values correlated well with the Ca and P content ( $r > 0.80$ ).

## **Conclusions**

Bleaching reduced the color disparities between sound and carious enamel without deteriorating the chemical and mechanical properties. The application of CPP-ACP paste enhanced mineral deposition in the subsurface lesion area of carious enamel.

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**Key Words:** Artificial caries, Bleaching, CPP-ACP, Color measurement, EPMA, Microhardness.

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## **I. Introduction**

Dental caries is a bacterial disease process caused by acids from bacterial metabolism and dissolves minerals on tooth surface. The cavitation of carious lesion is a result of continuous cycles of demineralization and remineralization.<sup>1</sup> Clinically, the initial caries lesion of the enamel is detected by white opaque discoloration of the enamel lesion exaggerating on dried condition. Initial caries lesion before cavitation has relatively intact surface layer and subsurface lesion which is low in mineral content. Further deterioration of the white spot lesion, carious lesion is visible without air drying.

Initial enamel caries causes subsurface demineralization underneath a superficially intact layer. Light is scattered differently on the surfaces of the demineralized enamel compared to the surrounding sound enamel, creating a chalky white appearance.<sup>1</sup> When white spot lesions are exposed to environments enhancing remineralization, additional minerals are adjoined to the superficial layer.<sup>2</sup> With a well-mineralized barrier, ionic ingress into the subsurface body lesion is hampered, resulting in only minimal alteration of the optical characteristics.<sup>3</sup> In clinical settings, the management of white spot lesions often involves removing the lesion and replacing it with a tooth-

colored restoration for esthetic improvement. However, this clinical intervention results in a cycle of repair and replacement of the restoration throughout an individual's lifetime.<sup>4</sup> The ideal management of a white spot lesion would be to enhance its physical appearance and reinforce its weakened substructure in a noninvasive manner.

The color changes in demineralized enamel are similar to those created by bleaching procedures, resulting in an increase in lightness and a decrease in yellowness.<sup>5,6</sup> Bleaching of the entire tooth structure containing the white spot lesion may provide a camouflage effect that makes the whiteness of the lesion less visible (Figure 1). However, the application of hydrogen peroxide to an already mineral-depleted part of the enamel may bring a potential concern for patients and dental practitioners. Previous studies have reported that bleaching peroxides change the calcium (Ca) and phosphate (P) content of enamel.<sup>7-9</sup> Hence, postbleaching treatment using remineralizing agents has been recommended for restoring the structural integrity of bleached enamel. Casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) is known to maintain a supersaturated mineral environment and induce remineralization at the tooth surface by stabilizing high concentrations of Ca and P ions.<sup>10,11</sup> However, clinicians may be uncertain as to whether

bleaching teeth with white spot lesions would have acceptable safety and efficacy.

In this *in vitro* study, artificial white spot lesions with standardized whiteness were produced on the surface of human premolars. The CIEL<sup>\*</sup>a<sup>\*</sup>b<sup>\*</sup> color values of the specimens were measured with a spectroradiometer at baseline, after the formation of the caries, after bleaching, and after remineralization. Electron probe microanalysis (EPMA) determined the weight percentages of Ca, P, and fluoride (F) in the cross-sectional surface of each specimen. A Knoop microhardness test was carried out along with each EPMA scan line to correlate the mineral content with the cross-sectional hardness of the lesion area. The null hypotheses tested in the study were that bleaching treatment using 10% carbamide peroxide on white spot lesions would not change the color, mineral content, or hardness of enamel and remineralization treatment using CPP-ACP paste would not affect these properties of white spot lesions with or without bleaching.

## **II. Materials & Methods**

### **II-1. Specimen Preparation**

Twenty human upper premolars extracted during dental treatments were used under the approval of the Institutional Review Board of Seoul National University Dental hospital (CRI13010). The teeth were disinfected in 0.5% chloramine-T for one week, stored in distilled water at 4°C less than 6 months, and then inspected under a 10× stereomicroscope (Jaemyung Ind, Seoul, Korea) to ensure that there were no white spot lesions or other defects. The roots of the teeth were removed at the cemento-enamel junction with a low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). The crown part was sectioned mesiodistally and buccolingually into four parts. Each quarter of the crown was ground on the dentin side, leaving enamel layer and a 2-mm-thick dentin layer, and was randomly distributed into four experimental groups. Group 1 was subdivided into 1A and 1B with 10 specimens allocated to each subgroup (Figure 2). The sections were then embedded in acrylic resin with a 2 × 4 mm window on the exposed enamel surface (Figure 3).

## **II-2. Artificial Carious Lesion Formation**

To form the artificial carious lesions for specimens in Groups 1B, 2, 3, and 4, pH cycling was applied three-times for 12 days. Each specimen was immersed in 2.5 mL of demineralizing solution (1.5 mM CaCl<sub>2</sub>, 0.9 mM KH<sub>2</sub>PO<sub>4</sub>, 50 mM acetate buffer, pH 4.8) for 72 hours, followed by immersion in 2.5 mL of remineralizing solution (1.5 mM CaCl<sub>2</sub>, 0.9 mM KH<sub>2</sub>PO<sub>4</sub>, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid [HEPES], pH 7.0) for 24 hours at 4°C with daily changes of the solution.

## **II-3. Bleaching and Remineralization Treatment**

To specimens in Group 1A (BL) and Group 3 (CA+BL), for the bleaching treatment, 10% carbamide peroxide gel (Opalescence Non-PF 10%, Ultradent, South Jordan, UT, USA) was applied on the exposed enamel surfaces. The surface was covered by a plastic mold made with 1 mm spacer (Sof-Tray Classic Sheets, Ultradent) and maintained for 8 hours. The specimens were washed with distilled water to remove the residual carbamide peroxide gel after bleaching and stored in artificial saliva for 16 hours. This daily bleaching procedure was repeated for 14 days. The pH of the bleaching gel used in the experiment was measured as 6.8.

During the remineralization procedure in Group 2 (CA+RE), F-containing CPP-ACP paste (Tooth Mousse Plus, GC, Tokyo, Japan) was applied on the enamel surface for 30 minutes twice a day for 14 days. After the completion of each application, the specimens were washed with distilled water and stored in artificial saliva. For the specimens in Group 4 (CA+BL+RE), CPP-ACP paste was applied to the specimen shortly after the bleaching gel was washed off.

#### **II-4. CIEL\*a\*b\* Color Measurement**

The color of the enamel surface was measured at baseline (BA), after formation of the white spot lesion (CA), and after completion of treatment (TR). For the color measurement, the specimens were retrieved from storage solution, dried with blotting paper, and immediately placed in a light booth (Color Sense II, Sungjin Hitech, Gyeonggi-Do, Korea) with Munsell N7 neutral gray walls and floor.

A spectroradiometer (PR-670 SpectraScan, Photo Research, Chatsworth, CA, USA) equipped with a Macro-Spectar MS-75 lens (Photo Research) was fixed on a tripod at a distance of 355 mm from the measured object and with a measurement area of 2.63 mm in diameter, providing an

optical configuration of 2° observation to the object. Four D65 simulating tubes (F2DT12/65, Gretagmacbeth, Research Triangle Park, NC, USA), reportedly having a correlated color temperature of 6500 K and a color rendering index of 91, were used as the light source. The tubes were bidirectionally fixed with a 45° illumination angle at a distance of 30 cm from the measured object. External light was excluded by covering the equipment with a light-proof cover (Figure 4). The positioning of the lens toward the surface of the specimen was kept constant to ensure a standardized measurement throughout the experiment. Spectral reflectance was obtained from 380 to 780 nm with a 2-nm interval (Spectrawin 2.0, Photo Research) and was subsequently converted to CIEL<sup>\*</sup>, *a*<sup>\*</sup>, and *b*<sup>\*</sup> values. The color difference ( $\Delta E^*$ ) was calculated by the equation:  $(\Delta E^*) = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ . Every measurement was performed after calibration over a white background and repeated three times.

## **II-5. Mineral content measurement using Electron probe microanalysis**

Specimens were embedded in epoxy resin (Epofix, Struers, Glasgow, UK) and cross-sectioned along the midline. The cut surfaces were serially polished with 1200-, 2400-, and 4000-grit silicon carbide abrasive papers, followed by 1- $\mu\text{m}$  and 0.25- $\mu\text{m}$  diamond and 0.1- $\mu\text{m}$  and 0.05- $\mu\text{m}$  aluminum oxide polishing suspensions (Struers, Copenhagen, Denmark). The specimens were ultrasonically cleaned in deionized water for 10 minutes, dried for 72 hours in a desiccator, and then sputter-coated with carbon. The demineralization area on the cross-sectioned enamel surfaces was identified using the phase contrast of the backscattered electron imaging mode of a scanning electron microscope (SEM, JEOL JSM-6610LV, JEOL). Two-line analyses were performed perpendicular to the outer enamel surface at 0.3- $\mu\text{m}$  pixel intervals. The observation areas (intact surface layer, demineralized subsurface layer, and inner sound enamel) were determined according to changes in calcium and phosphorus content using an electron microprobe (JEOL JXA-8100, JEOL). The operating conditions for the elemental analyses were 15 kV of accelerating voltage and 50 nA of beam current. A fluorapatite crystal (3.38% F) was used as a standard comparison for analysis.

## **II-6. Knoop Microhardness Measurement**

Cross-sectional enamel surface hardness was measured using a microhardness tester (Tukon 2100, Instron Corp, Canton, MA, USA), and a Knoop diamond indenter with a load of 10 g was applied for 11.5 seconds. A total of 10 indentations were made with a 20- $\mu\text{m}$  interval from the surface to the sound enamel along an EPMA scan line (Figure 5).

## **II-7. Statistical Analysis**

The sample size calculation was based on the data from a pilot study using the microhardness test and had 80% power to detect a 30  $H_k$ -standard deviation (SD) difference between any two groups, assuming an overall 5% significance level and two-sided tests. The normality and homogeneity of the samples were tested using the Kolmogorov-Smirnov test. Assuming a normal distribution of differences, two-way analysis of variance was performed with Tukey *post hoc* comparison. The mean values of  $CIEL^*$ ,  $a^*$ , and  $b^*$  and the differences between each measurement point were compared among the four groups. The mean percentage weight loss of Ca and P and the mean weight of F in the surface and the subsurface layer were compared among the groups. The mean values and percentage decreases of the

microhardness at the lesion areas were compared among the groups. The correlations between the Ca and P content and microhardness values were evaluated by Pearson correlation coefficients. A *p* value of 0.05 was selected as the threshold for statistical significance. Analyses were performed using SPSS 13.0 (SPSS, Chicago, IL, USA).

### III. Results

The CIEL<sup>\*</sup>*a*<sup>\*</sup>*b*<sup>\*</sup> values of the specimens at baseline (BA) and after caries formation (CA) were not significantly different among all groups. The final color change after treatment,  $\Delta E^*_{TR-BA}$  ranged from 7.03 to 7.60 in Groups 1A (BL), 1B (CA), and 2 (CA+RE), without any significant differences (Tables 1-4). However, the  $\Delta E^*_{TR-BA}$  values in Group 3 (CA+BL) and Group 4 (CA+BL+RE) were 10.98 and 10.81, respectively, which was significantly greater than those in the other three groups ( $p < 0.05$ ). The differences in the three color parameters,  $\Delta L^*_{TR-BA}$ ,  $\Delta a^*_{TR-BA}$ , and  $\Delta b^*_{TR-BA}$ , were in accordance with  $\Delta E^*_{TR-BA}$ ; each value in Groups 3 (CA+RE) and 4 (CA+BL+RE) was significantly greater than its counterpart in the other three groups ( $p < 0.05$ ).

The mean (SD) depth of the surface layer and subsurface lesion ranged from 22.3 (6.2)  $\mu\text{m}$  to 24.2 (7.9)  $\mu\text{m}$  and 153.7 (12.5)  $\mu\text{m}$  to 165.7 (18.4)  $\mu\text{m}$ , respectively, without any significant difference among all groups (Table 5). The amount of Ca and P loss in the subsurface lesion in Group 2 (CA+RE) was significantly less than that in Group 1B (CA) ( $p < 0.05$ ). The mean (SD) Ca/P ratios were 2.17 (0.10) and 2.15 (0.01) in the surface layer and subsurface lesion, respectively, without significant differences among groups. The weight of F was highest in the surface layer, followed by the subsurface lesion and the inner sound enamel across all groups ( $p < 0.05$ ,

Figure 7). The amount of F in the subsurface lesion was higher in Group 2 (CA+RE) than in Group 1B (CA) ( $p < 0.05$ ).

The mean (SD) Knoop microhardness value was 172.2 (49.6) to 201.0 (27.4)  $H_k$  in the surface layer, 288.7 (47.8) to 324.3 (46.2)  $H_k$  in the subsurface lesion, and 416.1 (32.5)  $H_k$  to 448.4 (49.2) in the sound enamel (Table 6). Each microhardness value of the surface, subsurface, and total lesion area did not show a significant difference among groups (Figure 8). A strong correlation existed between the Ca and P content and the microhardness values for the subsurface lesion area (Figure 9).

## IV. Discussion

For our first hypothesis, we primarily investigated whether the whiteness of carious enamel could be masked by the whitening effect of the surrounding sound enamel structure. The color change produced by the formation of artificial caries was similar to that obtained from peroxide bleaching. The increase in lightness ( $L^*$ ) and decrease in yellowness ( $b^*$ ) contributed to the whitening of artificial caries, which was in accordance with the results of previous studies.<sup>5,6</sup> The color of artificially formed carious enamel ( $\Delta E^* = 7.03$ ) was further changed by bleaching, resulting in extended whiteness ( $\Delta E^* = 10.98$ ). However, considering the outcome of bleaching sound enamel ( $\Delta E^* = 7.60$ ), the color discrepancy between the sound and carious enamel after bleaching ( $3.38 \Delta E^*$  units) was within a relatively acceptable range. In a widely cited study by Johnston and Kao,<sup>12</sup>  $3.7 \Delta E^*$  units was proposed as the perceptibility threshold and  $6.8 \Delta E^*$  units was proposed to be the borderline for color mismatch. Therefore, from an esthetic standpoint, the bleaching treatment of teeth containing white spot lesions may be a clinically relevant procedure to promote an optical camouflage effect.

Our second question for first hypothesis was whether bleaching treatment would cause further demineralization of the white spot lesion.

White spot lesions represent the preliminary stage of subsurface enamel demineralization, although they do not necessitate invasive restorative intervention. Hence, it is clinically meaningful to re-harden the surface enamel and maintain its mechanical integrity. We obtained the values at each location adjacent to the EPMA scan line in order to evaluate the depth-related chemical and mechanical properties.

We created a superficially intact enamel layer with a relatively uniform width of 22-24  $\mu\text{m}$ , in which the loss of Ca and P was minimal (0-2 wt%). The underlying subsurface area with mineral loss (15-25 wt%) had a depth of 150-160  $\mu\text{m}$ . This mineral-depleted porous substructure induced an altered light-scattering mode within the enamel structure, producing a whitish appearance. In Group 3 (CA+BL), bleaching of the carious enamel extended the whiteness ( $\Delta E^* = 3.38$ ) without additional mineral loss compared with Group 1b (CA) ( $p < 0.05$ ). The microhardness values of the surface, subsurface, and total lesion area did not show a significant difference between Group 1b (CA) and Group 3 (CA+BL). The hardness of the cross-sectional enamel gradually increased, with elevating mineral content reaching the level of the sound enamel.

The Ca and P content and hardness values were relatively well correlated throughout the lesion area. Only a small disparity existed at the

first 20- $\mu\text{m}$  surface area with decreased hardness. The surface of the enamel with the lesion was softened and porous but contained abundant mineral.<sup>13</sup> This mechanically weakened layer may be easily abraded by normal tooth brushing. In a clinical evaluation of initial caries,<sup>14</sup> the remission of whiteness was not entirely due to color reversal from the remineralized microstructure, but rather was due to mechanical removal of the superficially weakened layer.

Many previous studies have reported on the potential impact of bleaching on the enamel microstructure. It has been reported that bleaching treatment may decrease hardness, increase roughness of tooth surface. Such alterations are caused by a mineral loss of crystal structure after the bleaching procedure which reduce the calcium and phosphate ions, and modifying the morphology of crystals in the superficial layer.<sup>7-9</sup> Those results were influenced by many variables, such as tooth type, peroxide concentration, the pH of the bleaching agent, the duration of contact, and the treatment interval. In this study, bleaching using 10% carbamide peroxide with a pH of slightly less than 7 did not induce a significant mineral loss in carious enamel. Overall, bleaching using 10% carbamide peroxide did not deteriorate the chemical and mechanical properties of carious enamel. Our first null hypothesis was accepted.

To test the second hypothesis, we evaluated the effect of CPP-ACP on color, mineralization, and hardness of carious enamel before and after bleaching. CPP-ACP is known to provide high concentrations of calcium and phosphate ions by binding tooth surfaces. This CPP-ACP complex acts as a calcium and phosphate reservoir. On acidic challenge, attached CPP-ACP releases calcium and phosphate ions and maintain a state of supersaturation of these minerals which increase remineralization of carious lesion, as observed in other studies.<sup>10-11</sup>

Despite the confirmation of mineral gain in the subsurface lesion in Group 2 (CA+RE), no significant color reversal was detected by the spectroradiometer. This corresponds to a common clinical situation, with long-existing caries lesions arrested or regressed by well-mineralized surface enamel but still showing a whitish appearance. Even when some mineral deposition occurs in the underlying body lesion, the pore volume is decreased but the pore number is unchanged.<sup>2</sup> There are several stages of mineral deposition, including the formation and growth of new crystals and the regrowth of preexisting crystals.<sup>15</sup> Although the Ca/P ratio remains unchanged, the heterogeneity of the modified apatite structure contributes to the altered optical characteristics.<sup>16</sup> The color mismatch between the

remineralized and sound enamel substrate is unsolved, often requiring esthetic enhancement.

As for the remineralizing and rehardening effect of CPP-ACP on enamel during and after at-home bleaching procedures, many studies suffered from dissimilar experimental conditions; some studies used bovine teeth, which are more porous than human teeth,<sup>10,17</sup> while others measured the surface microhardness or roughness on the superficial enamel instead of its subsurface structure.<sup>11,18,19</sup> In this study, the change in mineralization was evaluated both at the surface and subsurface lesion areas after the use of CPP-ACP. We observed that carious enamel without bleaching had the largest remineralization gains. In Group 2 (CA+RE), freshly formed carious lesions, which were not subjected to a sequence of 10% carbamide application and artificial saliva storage, largely promoted incorporation of free ions from the CPP-ACP paste into the subsurface lesion area. The microhardness values were also highest in Group 2, both at the surface and subsurface lesion areas, and the values in other groups followed the same order as in the mineral composition. Overall, our second null hypothesis was not accepted.

Many previous studies<sup>9,20,21</sup> have determined the effect of remineralizing agents on bleached teeth and concluded that any substantial

recovery of hardness or mineral deposition was mainly due to supplementary ions in the storage media (artificial or human saliva). Under *in vivo* conditions, the repair mechanism would more actively counteract the mineral loss than under *in vitro* conditions, even in the case that the bleaching treatment might cause an initial deterioration of the chemical or mechanical properties of the enamel. We confirmed that the application of CPP-ACP paste enhanced the reversal of the early caries lesion stage, as shown in other studies.<sup>3,22</sup> Considering the largest change shown in Group 2, CPP-ACP's remineralizing effect on white spot lesions seemed to be maximized prior to the bleaching procedure.

## **V. Conclusions**

In this study, the 10% carbamide peroxide bleaching of enamel with white spot lesions decreased color disparities without deteriorating mineral composition or microhardness. The application of CPP-ACP paste promoted mineral gain in the subsurface body lesion. The bleaching treatment for teeth with white spot lesions can be recommended as a noninvasive esthetic treatment regimen with supplementary remineralization protocols.

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## VII. Tables & Figures

**Table 1.** Comparison of CIEL\* values at baseline (BA) after caries formation (CA), and after treatment (TR) among the experimental groups

<b>Group</b>	<b>N</b>	<b>BA</b>	<b>CA</b>	<b>TR</b>
<b>1A (BL)</b>	10	74.30 (1.31) <sup>a#</sup>	NA	79.23 (1.19) <sup>b</sup>
<b>1B (CA)</b>	10	75.20 (0.58) <sup>a</sup>	79.92 (0.79) <sup>b</sup>	NA
<b>2 (CA+RE)</b>	20	74.90 (0.98) <sup>a</sup>	79.58 (1.11) <sup>b</sup>	79.60 (1.17) <sup>b</sup>
<b>3 (CA+BL)</b>	20	74.73 (1.13) <sup>a</sup>	79.41 (1.27) <sup>b</sup>	81.70 (1.27) <sup>c</sup>
<b>4 (CA+BL+R E)</b>	20	74.96 (1.16) <sup>a</sup>	79.55 (1.27) <sup>b</sup>	81.82 (1.07) <sup>c</sup>

<sup>#</sup> Different superscript letters in a column denote significant differences at  $p < 0.05$ .

The numbers in the parenthesis are standard deviations.

NA: not applicable

BL : bleaching

RE : remineralization

**Table 2.** Comparison of CIEa\* values at baseline (BA) after caries formation (CA), and after treatment (TR) among the experimental groups

<b>Group</b>	<b>N</b>	<b>BA</b>	<b>CA</b>	<b>TR</b>
<b>1A (BL)</b>	10	1.65 (0.20) <sup>a#</sup>	NA	0.52 (0.16) <sup>c</sup>
<b>1B (CA)</b>	10	1.69 (0.17) <sup>a</sup>	1.18 (0.14) <sup>b</sup>	NA
<b>2 (CA+RE)</b>	20	1.70 (0.20) <sup>a</sup>	1.22 (0.19) <sup>b</sup>	1.24 (0.19) <sup>b</sup>
<b>3 (CA+BL)</b>	20	1.71 (0.20) <sup>a</sup>	1.22 (0.20) <sup>b</sup>	0.23 (0.18) <sup>c</sup>
<b>4 (CA+BL+RE)</b>	20	1.66 (0.19) <sup>a</sup>	1.17 (0.20) <sup>b</sup>	0.26 (0.15) <sup>c</sup>

<sup>#</sup> Different superscript letters in a column denote significant differences at  $p < 0.05$ .

The numbers in the parenthesis are standard deviations.

NA: not applicable

BL : bleaching

RE : remineralization

**Table 3.** Comparison of CIE*b*\* values at baseline (BA) after caries formation (CA), and after treatment (TR) among the experimental groups

<b>Group</b>	<b>N</b>	<b>BA</b>	<b>CA</b>	<b>TR</b>
<b>1A (BL)</b>	10	5.54 (1.01) <sup>a#</sup>	NA	-0.05 (1.04) <sup>b</sup>
<b>1B (CA)</b>	10	5.63 (1.18) <sup>a</sup>	0.57 (1.01) <sup>b</sup>	NA
<b>2 (CA+RE)</b>	20	5.48 (1.33) <sup>a</sup>	0.49 (0.99) <sup>b</sup>	0.21 (1.01) <sup>b</sup>
<b>3 (CA+BL)</b>	20	5.66 (1.16) <sup>a</sup>	0.11 (1.18) <sup>b</sup>	0.17 (1.15) <sup>b</sup>
<b>4 (CA+BL+RE)</b>	20	5.62 (1.11) <sup>a</sup>	0.13 (0.99) <sup>b</sup>	-2.64 (0.95) <sup>c</sup>

<sup>#</sup> Different superscript letters in a column denote significant differences at  $p < 0.05$ .

The numbers in the parenthesis are standard deviations.

NA: not applicable

BL : bleaching

RE : remineralization

**Table 4.** Comparison of CIE $\Delta L^*$ ,  $\Delta a^*$ ,  $\Delta b^*$ , and  $\Delta E^*$  at baseline (BA), after caries formation (CA), and after treatment (TR) among the experimental groups

Group	After caries formation (CA)				After treatment (TR)			
	$\Delta L^*$ (CA- BA)	$\Delta a^*$ (CA- BA)	$\Delta b^*$ (CA- BA)	$\Delta E^*$ (CA- BA)	$\Delta L^*$ (TR- BA)	$\Delta a^*$ (TR- BA)	$\Delta b^*$ (TR- BA)	$\Delta E^*$ (TR-BA)
<b>1A</b> <b>(BL)</b>	NA	NA	NA	NA	4.95 (0.55) <sup>a#</sup>	-1.13 (0.15) <sup>a</sup>	-5.60 (1.03) <sup>a</sup>	7.60 (0.84) <sup>a</sup>
<b>1B</b> <b>(CA)</b>	4.72 (0.54)	-0.50 (0.12)	-5.14 (0.95)	7.03 (0.90)	NA	NA	NA	7.03 (0.90) <sup>a</sup>
<b>2</b> <b>(CA+RE)</b>	4.67 (0.72)	-0.48 (0.11)	-5.36 (1.08)	7.18 (1.01)	4.69 (0.75) <sup>a</sup>	-0.46 (0.13) <sup>b</sup>	-5.30 (1.14) <sup>a</sup>	7.14 (1.09) <sup>a</sup>
<b>3</b> <b>(CA+BL)</b>	4.67 (0.67)	-0.48 (0.13)	-5.53 (1.00)	7.31 (0.78)	6.96 (0.75) <sup>b</sup>	-1.47 (0.24) <sup>c</sup>	-8.31 (0.94) <sup>b</sup>	10.98 (0.80) <sup>b</sup>
<b>4</b> <b>(CA+BL+RE)</b>	4.58 (0.75)	-0.49 (0.13)	-5.44 (0.97)	7.17 (1.01)	6.86 (0.69) <sup>b</sup>	-1.40 (0.23) <sup>c</sup>	-8.20 (0.86) <sup>b</sup>	10.81 (0.85) <sup>b</sup>

<sup>#</sup> Different superscript letters in a column denote significant differences at  $p < 0.05$ .

The numbers in the parenthesis are standard deviations.

NA: not applicable

BL : bleaching

RE : remineralization

**Table 5.** The depth ( $\mu\text{m}$ ) and weight loss (%) of Ca and P of the surface layer and the subsurface lesion

Group	Surface layer				Subsurface lesion			
	Depth ( $\mu\text{m}$ )	Loss of mineral contents (wt%)		Ca/P ratio	Depth ( $\mu\text{m}$ )	Loss of mineral contents (wt%)		Ca/P ratio
		Ca	P			Ca	P	
<b>1B</b> <b>(CA)</b>	24.2 (7.9)	2.6 (4.8)	1.5 (5.6)	2.24 (0.20)	164.2 (20.7)	25.2 (8.5) <sup>a#</sup>	24.8 (8.5) <sup>a</sup>	2.14 (0.08)
<b>2</b> <b>(CA+RE)</b>	23.9 (6.6)	0.0 (3.8)	1.4 (3.7)	2.17 (0.02)	153.7 (12.5)	16.2 (5.6) <sup>b</sup>	15.5 (6.0) <sup>b</sup>	2.14 (0.04)
<b>3</b> <b>(CA+BL)</b>	22.9 (5.4)	2.1 (4.8)	1.9 (3.0)	2.16 (0.03)	165.7 (18.4)	21.0 (7.2) <sup>ab</sup>	21.3 (7.1) <sup>ab</sup>	2.16 (0.03)
<b>4</b> <b>(CA+BL+RE)</b>	22.3 (6.2)	0.2 (1.9)	1.5 (1.1)	2.17 (0.02)	157.4 (24.8)	19.8 (6.7) <sup>ab</sup>	18.4 (5.7) <sup>ab</sup>	2.15 (0.03)

<sup>#</sup> Different superscript letters in a column denote significant differences at  $p < 0.05$ .

The numbers in the parenthesis are standard deviations.

CA: caries formation

BL : bleaching

RE : remineralization

**Table 6.** The microhardness and its percentile decrement of the surface layer and subsurface lesion

Group	N	Surface layer		Subsurface lesion		Total lesion		Sound enamel
		Micro-hardness (Hk <sup>†</sup> )	Decrement (%) <sup>‡</sup>	Micro-hardness (H <sub>k</sub> )	Decrement (%)	Micro-hardness (H <sub>k</sub> )	Decrement (%)	Micro-hardness (H <sub>k</sub> )
1B (CA)	10	172.2 (49.6) <sup>a</sup>	59.9 (16.1)	288.7 (47.8) <sup>b</sup>	34.6 (13.6)	274.1 (44.8) <sup>b</sup>	37.7 (13.6)	448.4 (49.2) <sup>c</sup>
2 (CA+RE)	10	201.0 (27.4) <sup>a</sup>	53.7 (8.1)	324.3 (46.2) <sup>b</sup>	25.8 (9.4)	308.9 (39.7) <sup>b</sup>	29.3 (8.2)	437.9 (34.2) <sup>c</sup>
3 (CA+BL)	10	175.8 (19.8) <sup>a</sup>	58.6 (5.7)	291.1 (86.9) <sup>b</sup>	32.7 (16.6)	276.7 (77.6) <sup>b</sup>	35.9 (14.8)	429.1 (43.6) <sup>c</sup>
4 (CA+BL+RE)	10	198.6 (18.1) <sup>a</sup>	51.9 (5.8)	292.4 (47.9) <sup>b</sup>	29.2 (12.8)	280.7 (41.7) <sup>b</sup>	32.1 (11.3)	416.1 (32.5) <sup>c</sup>

<sup>#</sup> Different superscript letters in a column denote significant differences at  $p < 0.05$ .

<sup>†</sup>  $H_k = 14.12P/\ell^2$  (kgf/mm<sup>2</sup>)

<sup>‡</sup> decrement (%) =  $[1 - (\text{microhardness value of each layer} / \text{microhardness value of inner sound enamel})] \times 100$

The numbers in the parenthesis are standard deviations.

CA: caries formation

BL : bleaching

RE : remineralization

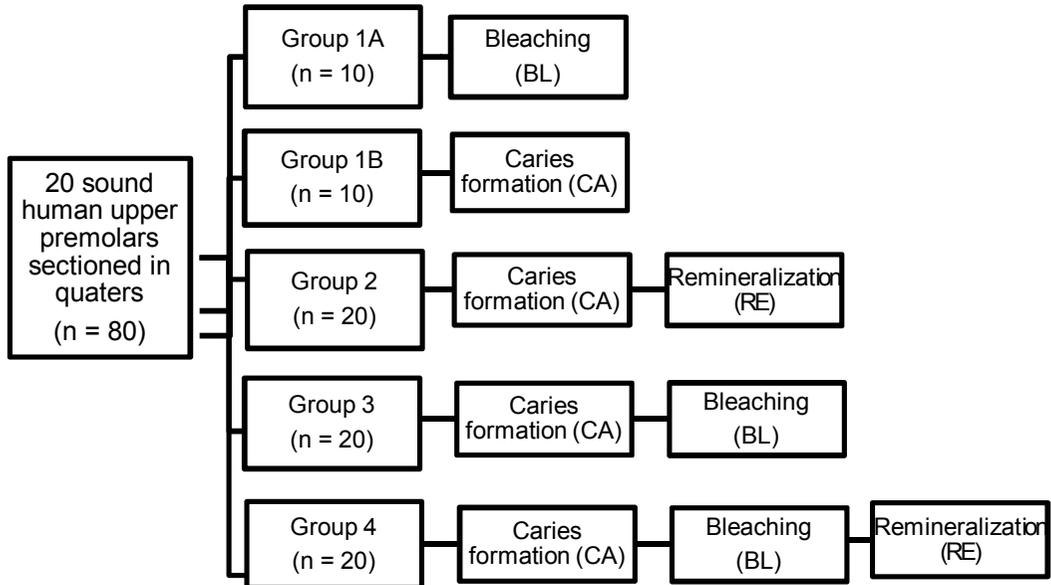


(a)



(b)

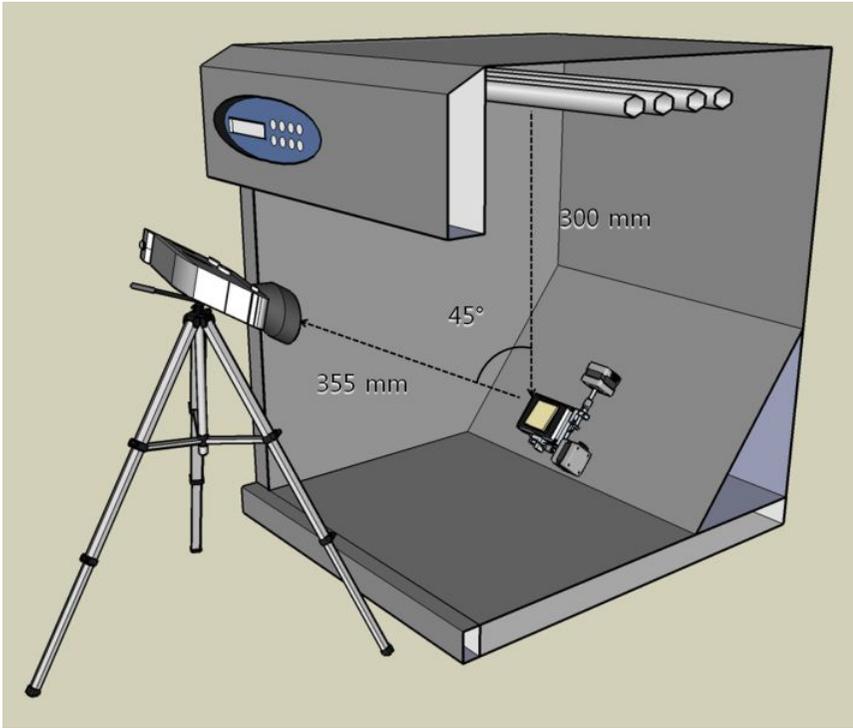
**Figure 1.** (a) Long-existing white spot lesions on the maxillary central incisors have a well-mineralized and hardened enamel surface not requiring a restorative treatment. (b) Bleaching enhanced overall whiteness in the upper dentition. White spot lesions became less noticeable after bleaching.



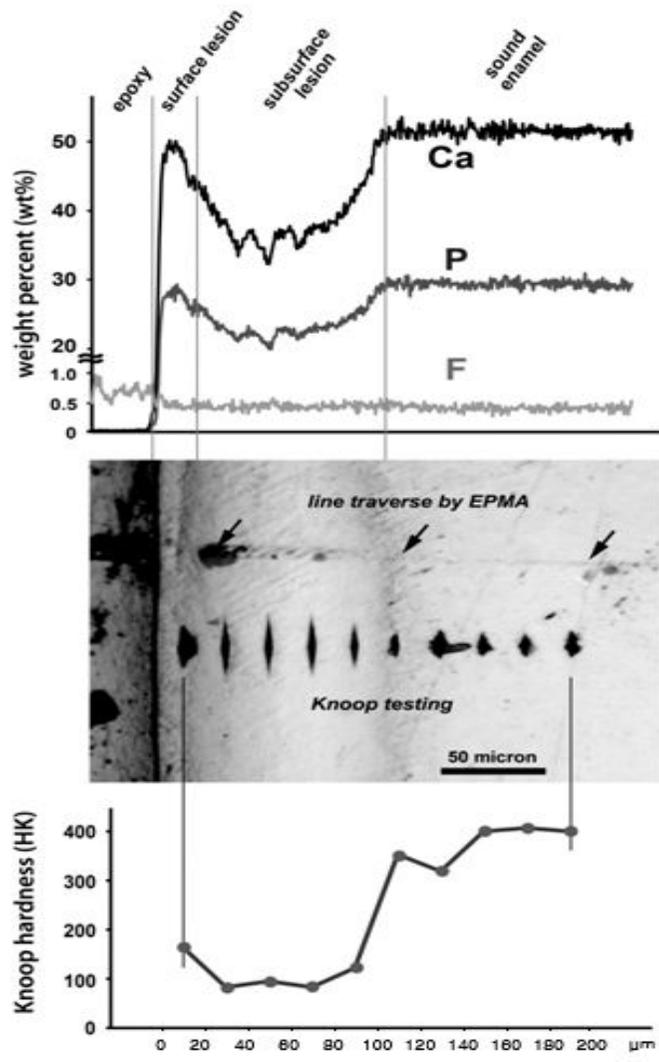
**Figure 2.** Procedural steps in four experimental groups.



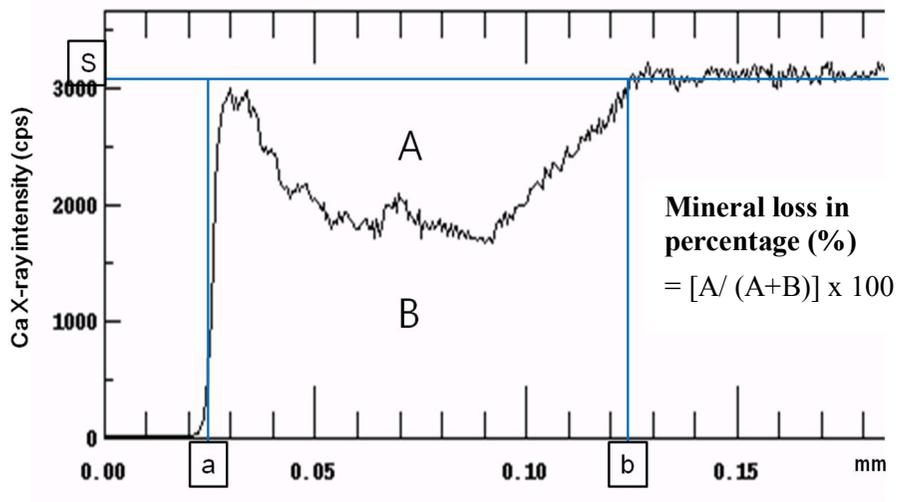
**Figure 3.** Specimen preparation. (A) Tooth was embedded in acrylic resin. (B) Four enamel surfaces with a size of 2 x 4 mm<sup>2</sup> were obtained from each tooth. (C) Specimen was embedded in acrylic resin which enamel surface was exposed.



**Figure 4.** Schematic diagram of the spectroradiometer used in the study.



**Figure 5.** Elemental analysis along the scan line (dark arrows) illustrated the elemental composition (Ca, P, and F) on the cross-sectional SEM image. Knoop microhardness values measured along the scan line corresponded to the elemental contents of each indentation site.



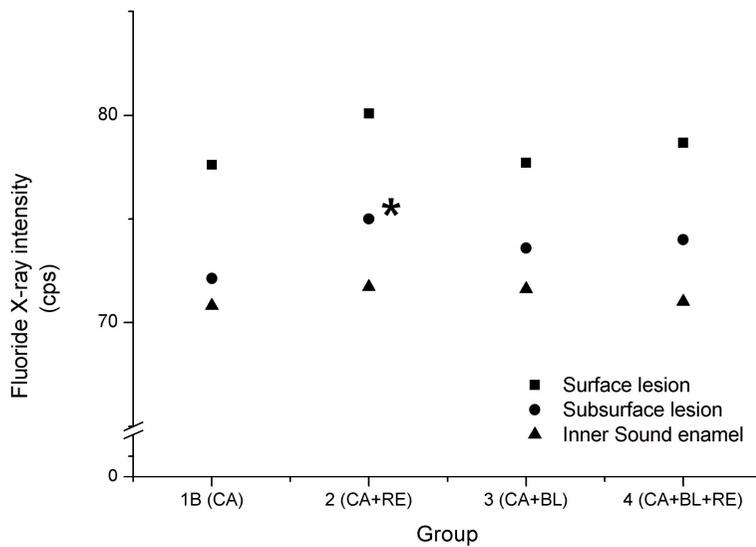
**Figure 6.** Schematic diagram of the calculation of mineral loss in percentage (total lesion area).

- a: initial point of surface layer
- b: terminal point of subsurface lesion area
- S: average of inner sound enamel

$$A = (b-a) \times S - \int_a^b (x - \text{ray intensity of ion})$$

$$B = \int_a^b (x - \text{ray intensity of ion})$$

cps: count per second



**Figure 7.** The mean content of F in each lesion area.

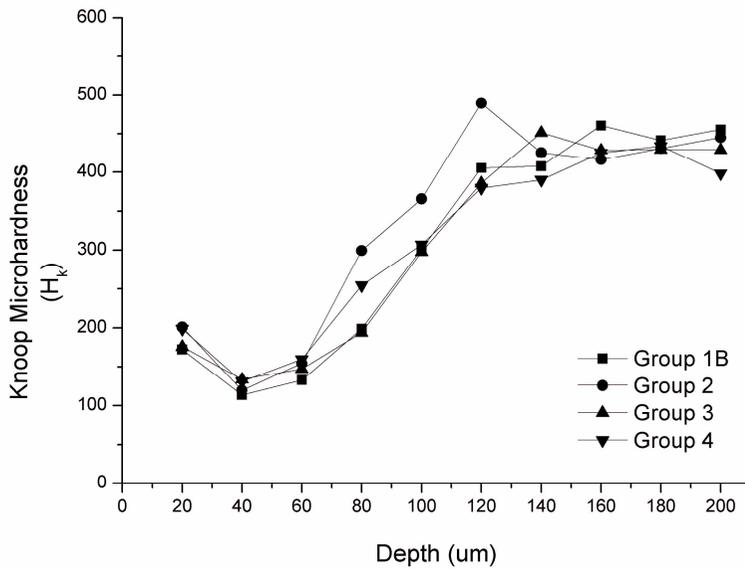
\* denote significant differences at  $p < 0.05$ .

CA: caries formation

BL : bleaching

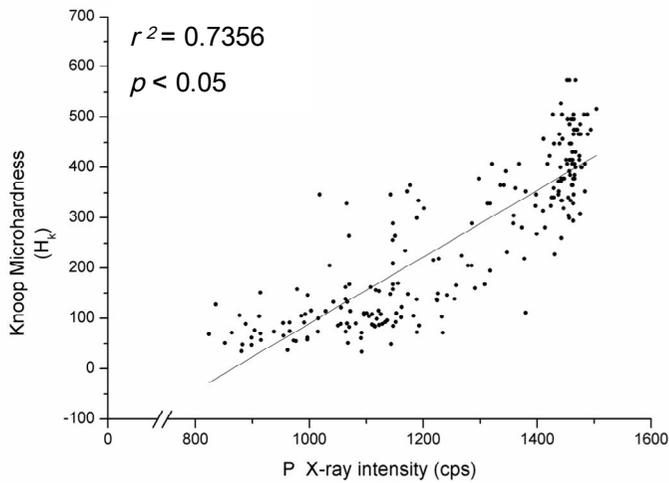
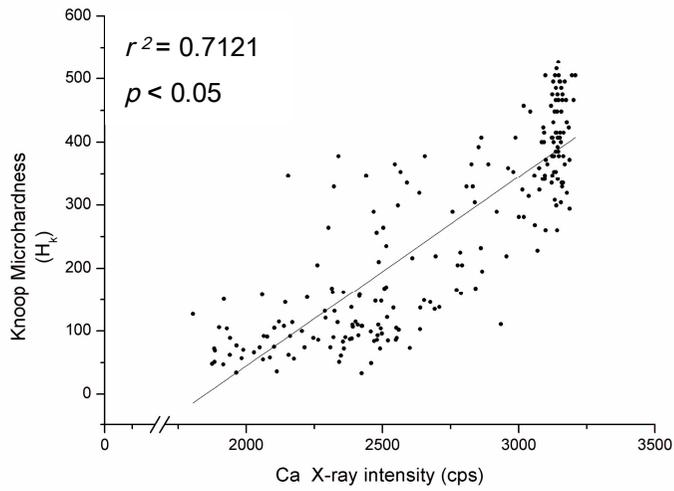
RE : remineralization

cps: count per second



**Figure 8.** The mean cross-sectional Knoop microhardness values of specimens from the four experimental groups. The Knoop microhardness values were measured at intervals of 20  $\mu\text{m}$  starting from the surface enamel layer and reaching to the 200- $\mu\text{m}$ -deep sound enamel.

$$H_k = 14.12P/\ell^2 (\text{kgf}/\text{mm}^2)$$



**Figure 9.** Correlation coefficients and regression equations between Ca and P contents and microhardness values in the subsurface lesion area of the carious enamel. ( $H_k=14.12P/\ell^2(\text{kg f/mm}^2)$ , cps: count per second)

국문초록

미백제 및 재광화제 사용이  
인공 초기 우식 병소의  
색 및 기계화학적 성질에 미치는 영향

김 연 주

서울대학교 대학원 치의과학과 치과보존학 전공

(지도교수: 손 호 현)

1. 목적

본 연구에서는 법랑질에 인공적으로 유발한 초기 우식 병소에 대해 미백제 및 재광화제를 적용하여 우식 병소의 색 및 기계화학적 성질의 변화를 관찰하였다.

## 2. 실험 재료 및 방법

20 개의 발거된 상악 소구치를 준비하여 치근부를 제거하고 협설 및 근원심으로 절단하여 치아당 4 개의 시편을 얻었다. 각 시편이 2 mm x 4 mm 의 법랑질이 노출되도록 매몰하고 다음 4 개의 실험군으로 나누었다.

실험군 1 은 대조군으로, 1A 와 1B 두 개의 하위 군으로 나누어 실험군 1A 는 10% carbamide peroxide gel 을 도포하고 8 시간 경과 후 인공 타액에 16 시간 보관하는 과정을 14 일간 반복하였다(BL). 실험군 1B 는 초기 우식 병소를 형성하기 위해 12 일간 pH cycling 을 시행하였다(artificial caries formation, CA). 실험군 2 는 인공우식 병소 형성 후 우식 표면에 불소를 함유한 Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) 를 하루 2 회 도포 후 수세하는 과정을 14 일간 반복하였다(CA+RE). 실험군 3 은 인공우식 병소 형성 후 우식 표면에 10% carbamide peroxide gel 을 도포하고 8 시간 경과 후 인공타액에 16 시간 보관하는 과정을 14 일간 반복하였다(CA+BL). 실험군 4 는 인공우식 병소 형성 후 10% carbamide peroxide bleaching 과 불소를 함유한 CPP-ACP 제재를 14 일간 적용하였다(CA+BL+RE).

법랑질 표면의 CIEL\**a*\**b*\* color scale 을 spectroradiometer (PR-670 SpectraScan, Photo Research, Chatsworth, CA, USA) 로 다음과 같이

3 차례 측정하였다. 1 차 - 건전한 법랑질 표면(baseline, BA); 2 차 - 인공우식 병소 형성 후(artificial caries formation, CA); 3 차 - 미백 과정 또는 CPP-ACP 적용 후(treatment, TR). 시편을 에폭시 레진에 매몰하고 횡절단하여 연은 단면을 연마하여 주사전자현미경의 backscattered electron imaging (BSI) mode 로 관찰한 뒤 법랑질의 표층우식 병소에서 내부 건전 법랑질까지의 Ca, P, F 의 함량 측정을 위해 Electron microprobe analysis (EPMA) 를 시행하였다. EPMA scan line 상에서 Knoop indenter 에 10 g 의 하중을 가해 20 micrometer 간격으로 횡단면의 미세경도를 측정하였다. 측정결과를 이원분산분석(two-way ANOVA) 과 Tukey 사후검정을 통해 분석하였다. Ca, P 중량과 횡단면의 미세경도 값은 Pearson 상관 계수를 계산하였다.

### 3. 실험결과

인공우식 병소 형성 전(baseline) 과 후(artificial caries formation) 실험군 간의 통계적으로 유의한 색차는 관찰되지 않았다. 법랑질 인공우식 병소 ( $\Delta E^* = 7.03$ ) 와 미백을 시행한 법랑질 ( $\Delta E^* = 7.60$ ) 의 건전 법랑질과의 색차는 통계적으로 유의한 차이를 보이지 않았다. 법랑질 인공우식 병소에 미백을 시행하였을 때 병소의 추가적인  $CIE L^*$  값의 증가와  $CIE b^*$  의 감소로 인한 인공우식 병소와의

색차( $\Delta E^* = 3.38$ ) 가 관찰되었으며( $p < 0.05$ ), 부가적인 탈회는 관찰되지 않았다. CPP-ACP 를 적용한 실험군 2 에서 표층 하부 병소의 Ca, P, F 이온이 통계적으로 유의하게 증가하였다( $p < 0.05$ ). 표층, 표층하부, 전체 병소의 횡단미세경도 측정값은 실험군 간 통계적으로 유의한 차이가 나타나지 않았다. 횡단미세경도 측정값과 Ca, P 함량은 높은 상관관계를 보였다( $r > 0.80$ ).

#### 4. 결론

미백은 부가적인 기계화학적 성질의 저하 없이 건전한 법랑질과 우식 법랑질의 색차를 감소시켰다. CPP-ACP 의 적용은 우식 법랑질의 표층 하부 병소의 재광화를 증진하였다.

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주요어 : 인공 우식, 미백, CPP-ACP, 색 측정, EPMA, 미세경도

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