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Bleaching Effects on Color, Chemical, and Mechanical Properties of White Spot Lesions

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Clinical Relevance

Bleaching initial enamel caries lesions with 10% carbamide peroxide can camouflage white spot lesions without affecting the chemical and mechanical properties of the enamel. Application of casein phosphopeptide–amorphous calcium phosphate is a possible supportive treatment to promote remineralization of caries lesions.

SUMMARY

Objective: The purpose of the study was to evaluate the effect of bleaching on teeth with white spot lesions.

Methods and Materials: 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/group) as follows: group 1, control; group 2, caries formation followed by remineralization using fluoride-containing casein phosphopeptide–amorphous calcium phosphate (CPP-ACP; Tooth Mousse Plus, GC, Tokyo, Japan); group 3, caries formation followed by bleaching using 10% carbamide peroxide; and group 4, caries formation followed by both bleaching and remineralization. The CIE $L^*a^*b^*$ color 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 hardness test was carried out along the EPMA scan line. Two-way analysis of variance was performed with Tukey *post hoc* comparison.

Results: The change in the CIE color values was not significantly different between the caries-formed (ΔE^*=7.03) and the bleached enamel (ΔE^*=7.60). Bleaching of the carious enamel extended the whiteness (ΔE^*=3.38) without additional mineral loss ($p<0.05$). The remineralization treatment significantly increased the calcium (Ca), phosphate (P), and fluoride content of the subsurface lesion area ($p<0.05$). The cross-sectional microhardness

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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.

INTRODUCTION

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.[1] When white spot lesions are exposed to environments enhancing remineralization, additional minerals are adjoined to the superficial layer.[2] With a well-mineralized barrier, ionic ingress into the subsurface body lesion is hampered, resulting in only minimal alteration of the optical characteristics.[3] 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.[4]

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.[5,6] 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 agents 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.[7-9] 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.[10,11] 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 CIE $L^*a^*b^*$ 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 1) bleaching treatment using 10% carbamide peroxide on white spot lesions would not change the color, mineral content, or hardness of enamel and 2) remineralization treatment using CPP-ACP paste would not affect these properties of white spot lesions with or without bleaching.

METHODS AND MATERIALS

Specimen Preparation

Twenty human upper premolars extracted during dental treatments were used under the approval of the local Institutional Review Board. The teeth were disinfected in 0.5% chloramine-T for one week, stored in distilled water at 4°C, 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 cementoenamel 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 a 2-mm-thick dentin layer, and was randomly distributed into four experimental groups (Figure 2). The sections were then embedded in acrylic resin with a 2×4 mm window on the exposed enamel surface.

Artificial Caries Lesion Formation

To form the artificial caries lesions, pH cycling was applied three-times (12 days). Each specimen was immersed in 2.5 mL of demineralizing solution (1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 50 mM acetate buffer, pH 4.8) for 72 hours, followed by immersion in 2.5 mL of remineralizing solution (1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid [HEPES], pH 7.0) for 24 hours at 4°C with daily changes of the solution.

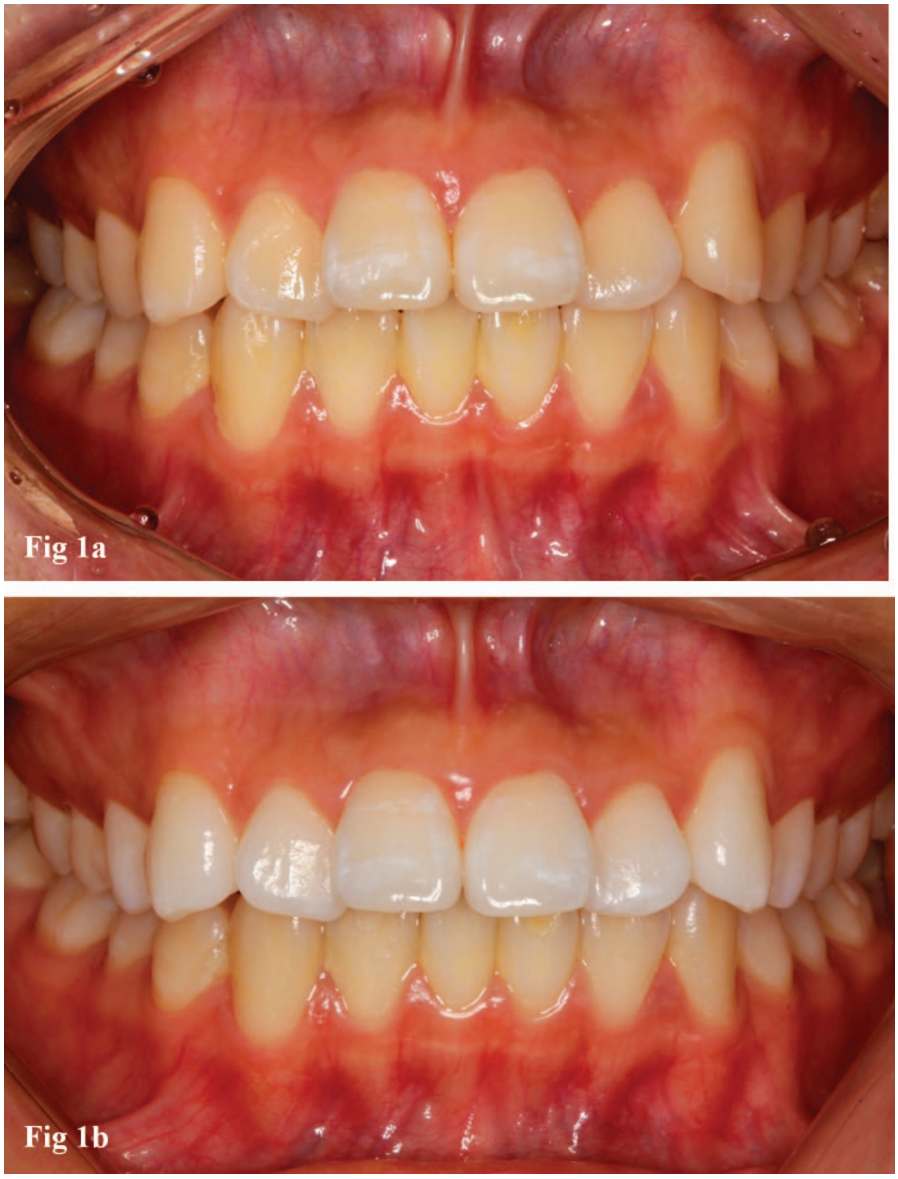


Figure 1. *(a) Long-existing white spot lesions on the maxillary central incisors have a well-mineralized and hardened enamel surface that does not require a restorative treatment. (b) Bleaching enhanced overall whiteness in the upper dentition. White spot lesions became less noticeable after bleaching.*

Bleaching and Remineralization Treatment

For the bleaching treatment, 10% carbamide peroxide gel (Opalescence Non-PF 10%, Ultradent, South Jordan, UT, USA) was applied on the exposed enamel surfaces with the aid of a plastic mold to create a 1 mm thickness (Sof-Tray Classic Sheets, Ultradent) and maintained for eight 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, 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, CPP-ACP paste was applied to the specimen shortly after the bleaching gel was washed off.

CIE *L*a*b** Color Measurement

The color of the enamel surface was measured at baseline, after formation of the white spot lesion, and after completion of bleaching and/or remineralization. For the color measurement, the specimens were retrieved from storage solution, dried with blotting paper, and immediately placed in a light booth (Color

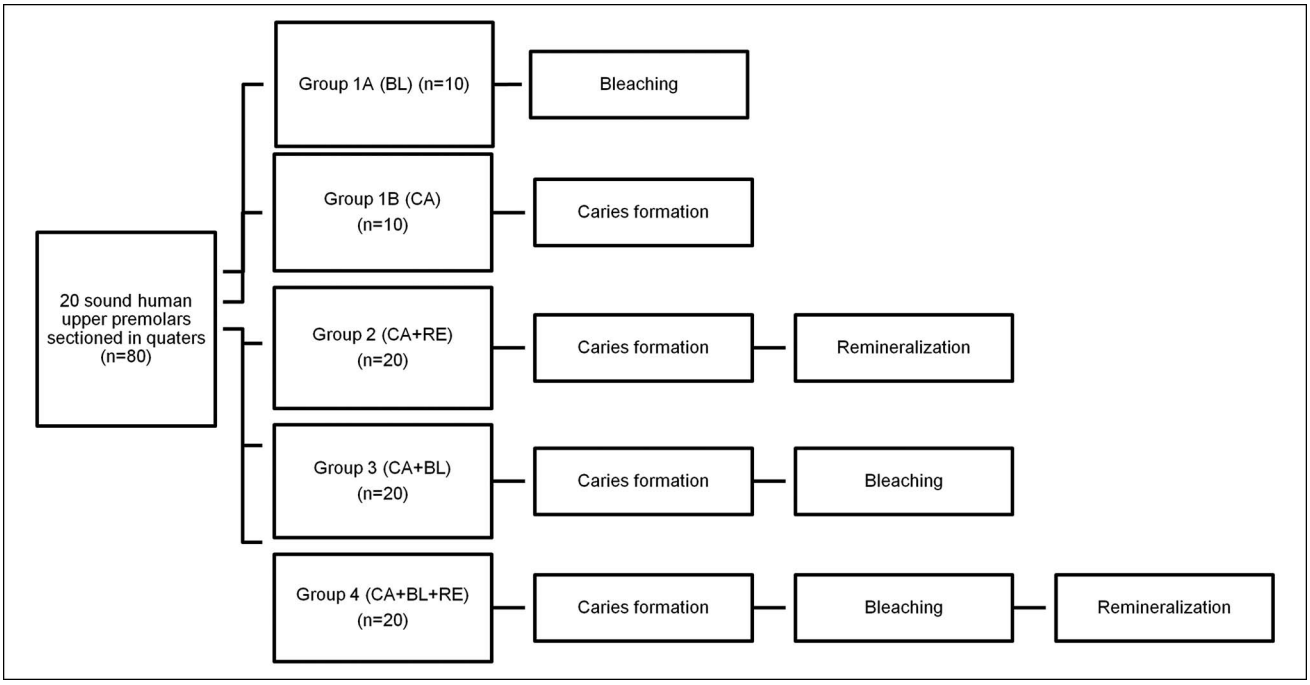


Figure 2. *Procedural steps in four experimental groups.*

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. 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 CIE L^*, a^*, and b^* values. The color difference (ΔE^*) 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.

EPMA

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-μm and 0.25-μm diamond and 0.1-μm and 0.05-μm 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, Akishima, Japan). Two-line analyses were performed perpendicular to the outer enamel surface at 0.3-μm pixel intervals. The observation areas (intact surface layer, demineralized subsurface layer, and inner sound enamel) were determined according to changes in Ca and P content using an electron microprobe (JEOL JXA-8100, JEOL, Akishima, Japan). 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.

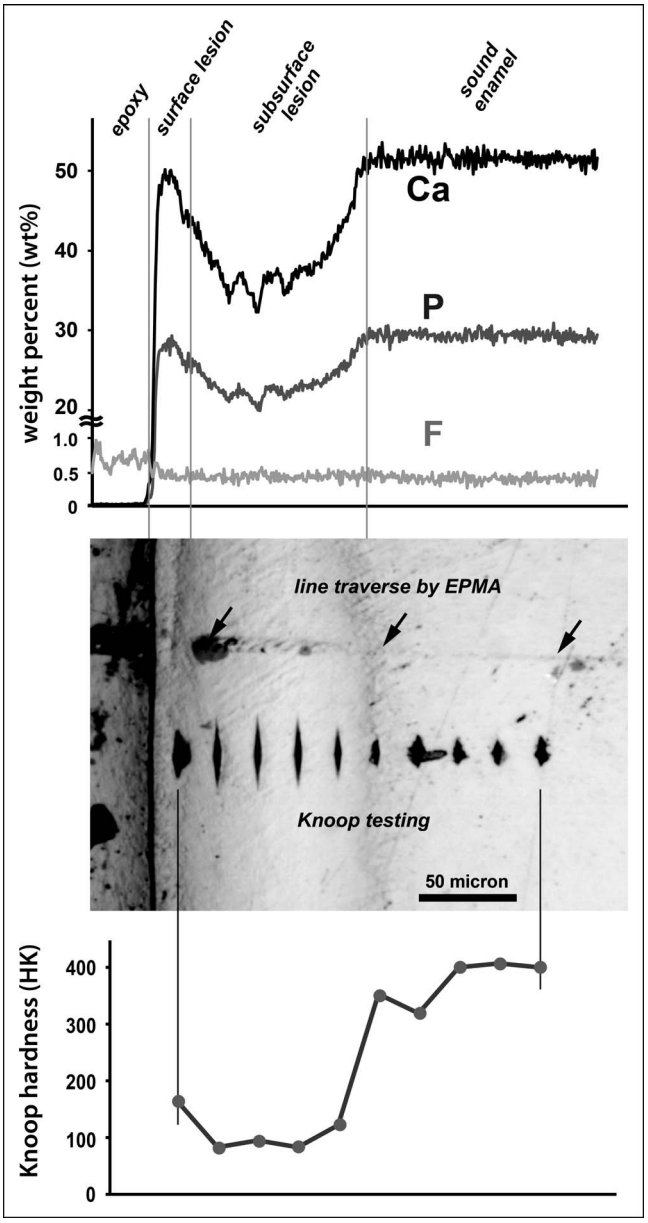


Figure 3. *Elemental analysis along the scan line (dark arrows) illustrated the elemental composition (Ca, P, and F) on the cross-sectional SEM image. Knoop microhardness values measured along the scan line corresponded to the elemental contents of each indentation site.*

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-μm interval from the surface to the sound enamel alongside an EPMA scan line (Figure 3).

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–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 CIE L^*, 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).

RESULTS

The CIE $L^*a^*b^*$ values of the specimens at baseline (BA) and after caries formation were not significantly different among all groups. The final color change after treatment of bleaching, remineralization (RE) and/or bleaching (BL), $\Delta E^*_{\text{TR-BA}}$, ranged from 7.03 to 7.60 in groups 1A (BL), 1B (CA), and 2 (CA+RE), without any significant differences (Table 1). However, the $\Delta E^*_{\text{TR-BA}}$ 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, $\Delta L^*_{\text{TR-BA}}$, $\Delta a^*_{\text{TR-BA}}$, and $\Delta b^*_{\text{TR-BA}}$, were in accordance with $\Delta E^*_{\text{TR-BA}}$; each value in groups 3 (CA+RE) and 4 (CA+BL+RE) was significantly larger than its counterpart in the other three groups ($p<0.05$).

The mean (SD) depth of the surface and subsurface lesion areas ranged from 23.4 (5.8) μm to 157.8 (19.0) μm, respectively, without any significant difference among all groups (Table 2). 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 and subsurface lesion areas, respectively, without significant differences among groups. The weight of F was highest in the surface lesion, followed by the

Table 1: *Comparison of the Mean (SD) Values of Color Difference Between at Baseline (BA) and After Treatment (TR) Among the Experimental Groups*

Group: Treatment	N	Color Difference Between at Basement and After Treatment[a]			
		$\Delta L^*_{(TR-BA)}$	$\Delta a^*_{(TR-BA)}$	$\Delta b^*_{(TR-BA)}$	$\Delta E^*_{(TR-BA)}$
1A (BL)	10	4.95 (0.55) A	−1.13 (0.15) A	−5.60 (1.03) A	7.60 (0.84) A
1B (CA)	10	4.72 (0.54) A	−0.50 (0.12) A	−5.14 (0.95) A	7.03 (0.90) A
2 (CA + RE)	20	4.69 (0.75) A	−0.46 (0.13) B	−5.30 (1.14) A	7.14 (1.09) A
3 (CA + BL)	20	6.96 (0.75) B	−1.47 (0.24) C	−8.31 (0.94) B	10.98 (0.80) B
4 (CA + BL + RE)	20	6.86 (0.69) B	−1.40 (0.23) C	−8.20 (0.86) B	10.81 (0.85) B

Abbreviations: BL, bleaching; CA, caries formation; RE, remineralization.
[a] *Different superscript letters following means denote significant differences (*p<0 .05).

subsurface lesion and the inner sound enamel across all groups ($p<0.05$, Figure 4). 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 231.3 (99.3) H_k in the surface lesion, 324.5 (76.0) H_k in the subsurface lesion, and 426 (38.7) H_k in the sound enamel (Table 3). Each microhardness value of the surface, subsurface, and total lesion area did not show a significant difference across groups (Figure 5). A strong correlation existed between the Ca and P content and the microhardness values for the subsurface lesion area (Figure 6).

DISCUSSION

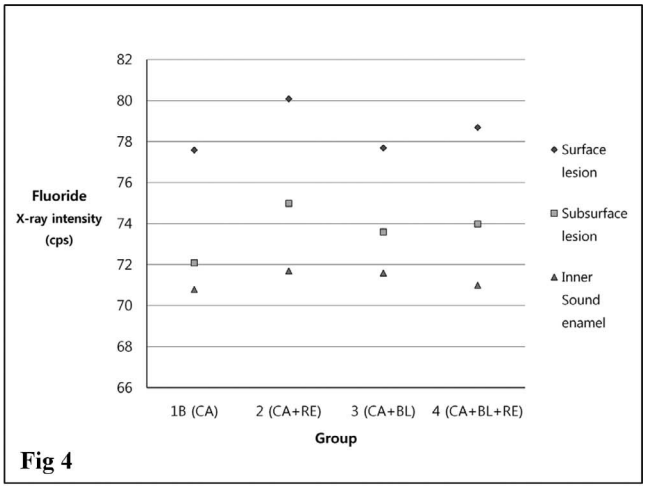
For our first hypothesis, we primarily investigated whether the whiteness of carious enamel could be masked by the whitening effect of the surrounding sound enamel structure. The color change produced by the formation of artificial caries was similar to that obtained from peroxide bleaching. The increase 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.[5,6] 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 ΔE^* units) was within a relatively acceptable range. In a widely cited study by Johnston and Kao,[12] 3.7 ΔE^* units was proposed as the perceptibility threshold and 6.8 ΔE^* 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 was whether bleaching treatment would cause further demineralization of the white spot lesion. We created a superficially intact enamel layer with a relatively uniform width of 22-24 μm, 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 μm. This mineral-depleted porous substructure induced an altered light-scattering mode within the enamel structure, producing a whitish appearance. Many previous studies have reported on the potential impact of bleaching on the enamel microstructure; 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,

Table 2: *The Mean (SD) Depth (μm) and Weight Loss (%) of Calcium (Ca) and Phosphorus (P) on the Surface and the Subsurface Lesions*[a]

Group (Treatment)	N	Surface Lesion			Subsurface Lesion		
		Depth, μm	Ca Loss, wt%	P Loss, wt%	Depth, μm	Ca Loss, wt%	P Loss, wt%
1B (CA)	10	24.2 (7.9)	2.6 (4.8)	1.5 (5.6)	164.2 (20.7)	25.2 (8.5) A	24.8 (8.5) A
2 (CA+RE)	10	23.9 (6.6)	0 (3.8)	1.4 (3.7)	153.7 (12.5)	16.2 (5.6) B	15.5 (6.0) B
3 (CA+BL)	10	22.9 (5.4)	2.1 (4.8)	1.9 (3.0)	165.7 (18.4)	21 (7.2) AB	21.3 (7.1) AB
4 (CA+BL+RE)	10	22.3 (6.2)	0.2 (1.9)	1.5 (1.1)	157.4 (24.8)	19.8 (6.7) AB	18.4 (5.7) AB

Abbreviations: BL, bleaching; Ca, calcium; CA, caries formation; P, phosphorus; RE, remineralization.
[a] *Different superscript letters following means denote significant differences at* p < 0.05.



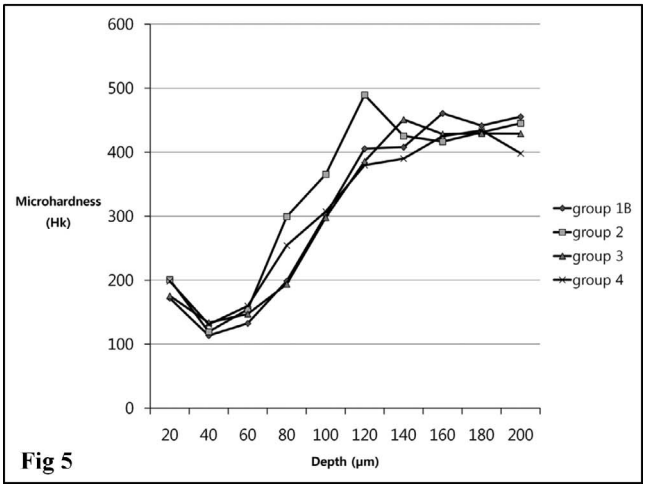
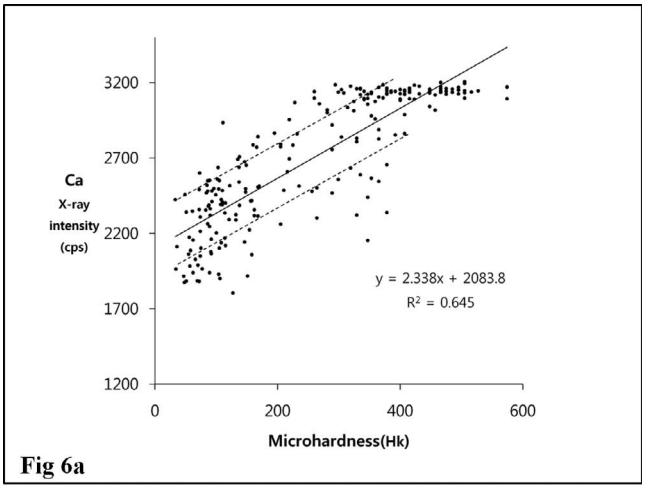


Figure 4. *The mean content of F in each lesion area.*
Figure 5. *The mean cross-sectional Knoop microhardness values of specimens from the four experimental groups. The Knoop microhardness values were measured at intervals of 20 μm starting from the surface enamel layer and reaching to the 200-μm-deep sound enamel.*



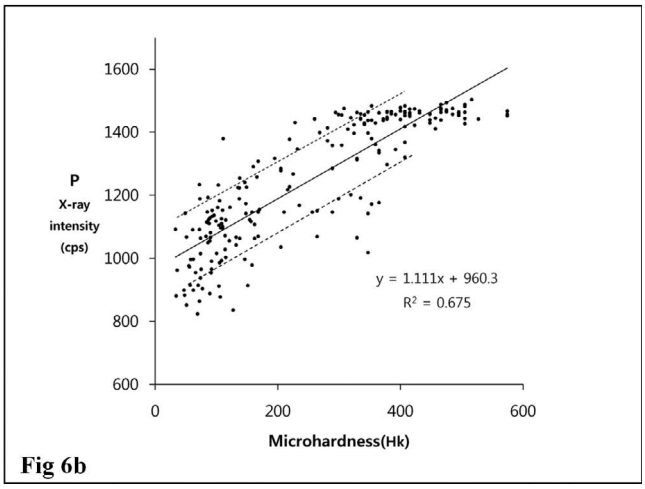


Figure 6. *Correlation coefficients and regression equations between Ca and P contents and microhardness values in the subsurface lesion area of the carious enamel.*

bleaching using 10% carbamide peroxide with a pH of slightly less than 7 did not induce a significant mineral loss in carious enamel. We obtained the cross-sectional microhardness values at each location adjacent to the EPMA scan line in order to evaluate the depth-related chemical and mechanical properties. 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 reharden the surface enamel and maintain its mechanical integrity. The Ca and P content and

Table 3: *The Mean (SD) Microhardness and Percentile Decrease of the Surface and Subsurface Lesions*

Group (Treatment)	N	Surface Lesion		Subsurface Lesion		Total Lesion	
		Microhardness, H_k[a]	Decrement, %[b]	Microhardness, H_k	Decrement, %	Microhardness, H_k	Decrement, %
1B (CA)	10	172.2 (49.6)	59.9 (16.1)	288.7 (47.8)	34.6 (13.6)	274.1 (44.8)	37.7 (13.6)
2 (CA+RE)	10	201.0 (27.4)	53.7 (8.1)	324.3 (46.2)	25.8 (9.4)	308.9 (39.7)	29.3 (8.2)
3 (CA+BL)	10	175.8 (19.8)	58.6 (5.7)	291.1 (86.9)	32.7 (16.6)	276.7 (77.6)	35.9 (14.8)
4 (CA+BL+RE)	10	198.6 (18.1)	51.9 (5.8)	292.4 (47.9)	29.2 (12.8)	280.7 (41.7)	32.1 (11.3)

Abbreviations: BL, bleaching; CA, caries formation; RE, remineralization.
[a] $H_k = 14.12P/\ell^2$ (kgf/mm^2).
[b] *Decrement (%) = (1−the mean microhardness value of each lesion area/the mean of the inner sound enamel) × 100.*

hardness values were relatively well correlated throughout the lesion area. Only a small disparity existed at the first 20-μm surface area with decreased hardness. The surface of the enamel with the lesion was softened and porous but mineral-abundant.[13] This mechanically weakened layer may be easily abraded by normal tooth brushing. In a clinical evaluation[14] of initial caries, 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. The hardness of the cross-sectional enamel gradually increased, with elevating mineral content reaching the level of the sound enamel. Overall, bleaching using 10% carbamide peroxide did not deteriorate the chemical and mechanical properties of carious enamel. Our first hypothesis was accepted.

To test our second hypothesis, we evaluated the effect of CPP-ACP on color, mineralization, and hardness of carious enamel before and after bleaching. Despite confirmation of mineral gain in the subsurface lesion, 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.[2] There are several stages of mineral deposition, including the formation and growth of new crystals and the regrowth of preexisting crystals.[15] Although the Ca/P ratio remains unchanged, the heterogeneity of the modified apatite structure contributes to the altered optical characteristics.[16] 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,[10,17] while others measured the surface microhardness or roughness on the superficial enamel instead of its subsurface structure.[11,18,19] In this study, the change in mineralization was evaluated both at the surface and subsurface lesion areas after the use of CPP-ACP. We determined that carious enamel without bleaching had the largest remineralization gains. In group 2, 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 hypothesis was not accepted.

Many previous studies[9,20,21] 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.[3,22] 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.

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.

Acknowledgement

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of Seoul National University Dental Hospital Institutional Review Board. The approval code for this study is CRI13010.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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REFERENCES

1. Kleter GA (1998) Discoloration of dental carious lesions (a review) *Archives of Oral Biology* **43(8)** 629-632.
2. Jones RS, & Fried D (2006) Remineralization of enamel caries can decrease optical reflectivity *Journal of Dental Research* **85(9)** 804-808.
3. Bailey DL, Adams GG, Tsao CE, Hyslop A, Escobar K, Manton DJ, Reynolds EC, & Morgan MV (2009) Regression of post-orthodontic lesions by a remineralizing cream *Journal of Dental Research* **88(12)** 1148-1153.
4. Mjor IA, & Gordan VV (2002) Failure, repair, refurbishing and longevity of restorations *Operative Dentistry* **27(5)** 528-534.
5. Iwami Y, Hayashi N, Takeshige F, & Ebisu S (2008) Relationship between the color of carious dentin with varying lesion activity, and bacterial detection *Journal of Dentistry* **36(2)** 143-151.
6. Kim Y, Son HH, Yi K, Kim HY, Ahn J, & Chang J (2013) The color change in artificial white spot lesions measured using a spectroradiometer *Clinical and Oral Investigations* **17(1)** 139-146.
7. Yeh ST, Su Y, Lu YC, & Lee SY (2005) Surface changes and acid dissolution of enamel after carbamide peroxide bleach treatment *Operative Dentistry* **30(4)** 507-515.
8. Joiner A (2007) Review of the effects of peroxide on enamel and dentine properties *Journal of Dentistry* **35(12)** 889-896.
9. Attin T, Schmidlin PR, Wegehaupt F, & Wiegand A (2009) Influence of study design on the impact of bleaching agents on dental enamel microhardness: A review *Dental Materials* **25(2)** 143-157.
10. Borges BC, Borges JS, de Melo CD, Pinheiro IV, Santos AJ, Braz R, & Montes MA (2011) Efficacy of a novel at-home bleaching technique with carbamide peroxides modified by CPP-ACP and its effect on the microhardness of bleached enamel *Operative Dentistry* **36(5)** 521-528.
11. de Vasconcelos AA, Cunha AG, Borges BC, Vitoriano Jde O, Alves-Junior C, Machado CT, & dos Santos AJ (2012) Enamel properties after tooth bleaching with hydrogen/carbamide peroxides in association with a CPP-ACP paste *Acta Odontologica Scandinavica* **70(4)** 337-343.
12. Johnston WM, & Kao EC (1989) Assessment of appearance match by visual observation and clinical colorimetry *Journal of Dental Research* **68(5)** 819-822.
13. Arends J, & Christoffersen J (1986) The nature of early caries lesions in enamel *Journal of Dental Research* **65(1)** 2-11.
14. Kielbassa AM, Gillmann L, Zantner C, Meyer-Lueckel H, Hellwig E, & Schulte-Monting J (2005) Profilometric and microradiographic studies on the effects of toothpaste and acidic gel abrasivity on sound and demineralized bovine dental enamel *Caries Research* **39(5)** 380-386.
15. Kawasaki K, Tanaka Y, & Takagi O (2000) Crystallographic analysis of demineralized human enamel treated by laser-irradiation or remineralization *Archives of Oral Biology* **45(9)** 797-804.
16. Ko CC, Tantbirojn D, Wang T, & Douglas WH (2000) Optical scattering power for characterization of mineral loss *Journal of Dental Research* **79(8)** 1584-1589.
17. Bayrak S, Tunc ES, Sonmez IS, Egilmez T, & Ozmen B (2009) Effects of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) application on enamel microhardness after bleaching *American Journal of Dentistry* **22(6)** 393-396.
18. Pinto CF, Paes Leme AF, Cavalli V, & Giannini M (2009) Effect of 10% carbamide peroxide bleaching on sound and artificial enamel carious lesions *Braz Dent J* **20(1)** 48-53.
19. De Abreu DR, Sasaki RT, Amaral FL, Florio FM, & Basting RT (2011) Effect of home-use and in-office bleaching agents containing hydrogen peroxide associated with amorphous calcium phosphate on enamel microhardness and surface roughness *Journal of Esthetic and Restorative Dentistry* **23(3)** 158-168.
20. Metz MJ, Cochran MA, Matis BA, Gonzalez C, Platt JA, & Pund MR (2007) Clinical evaluation of 15% carbamide peroxide on the surface microhardness and shear bond strength of human enamel *Operative Dentistry* **32(5)** 427-436.
21. Smidt A, Feuerstein O, & Topel M (2011) Mechanical, morphologic, and chemical effects of carbamide peroxide bleaching agents on human enamel in situ *Quintessence International* **42(5)** 407-412.
22. Cai F, Manton DJ, Shen P, Walker GD, Cross KJ, Yuan Y, Reynolds C, & Reynolds EC (2007) Effect of addition of citric acid and casein phosphopeptide-amorphous calcium phosphate to a sugar-free chewing gum on enamel remineralization in situ *Caries Research* **41(5)** 377-383.