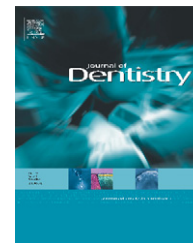


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Effect of fluoride containing bleaching agents on enamel surface properties

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ABSTRACT

Objectives: To evaluate the effects of fluoridated bleaching agents and post-bleaching fluoridation treatment on the whitening efficiency and microhardness of bovine enamel.

Methods: Twenty five freshly extracted bovine incisors were cut into halves, embedded and then divided into the following five groups: Group 1, untreated controls; Group 2, treatment with 10% carbamide peroxide (CP) bleaching agent; Group 3, treatment with 10% CP followed by a 0.9% sodium fluoride gel application, Group 4, treatment with 10% CP containing 0.11% fluoride; Group 5, treatment with an experimental bleaching agent consisting of 10% CP and 0.37% fluoride. Groups 2–5 were treated 8 h per day for 14 days then immersed in saliva for 2 weeks. Enamel morphology changes were evaluated under SEM on Day 14. Changes in enamel color and microhardness were evaluated on Days 7 and 14, and compared with the baseline data. Additionally, microhardness was determined on post-bleaching Days 21 and 28.

Results: After 2 weeks, an erosion pattern was noted on the specimens in Groups 2 and 3. Groups 4 and 5 showed a milder demineralized pattern. All the bleached enamel specimens revealed increased whiteness and overall color value. Groups 2 and 3 showed significantly decreased enamel microhardness compared to their baseline data. The specimens treated with fluoridated bleaching agents showed relatively less reduction in enamel microhardness than those treated with nonfluoridated agents during the bleaching treatment.

Conclusions: The fluoridated bleaching agents produced less demineralization of surface morphology and microhardness. The addition of fluoride did not impede the whitening effect.

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1. Introduction

With the increasing demand for treatments to enhance aesthetic appearance, tooth bleaching is becoming a more common procedure in dental clinics. Intrinsic tooth discoloration is caused by incorporation of chromogenic material into dentin and enamel during the tooth development stage or after eruption. Current vital and nonvital bleaching

techniques employ oxidizing agents such as hydrogen peroxide or peroxide releasing agents to brighten teeth. Popularity of these bleaching procedures increased following approval of their safety.¹

The different compositions and concentrations of bleaching agents for clinical use give dentists many options when prescribing tooth whitening. Carbamide peroxide (CP) is a perhydrol-urea and hydrogen peroxide carbamide compound

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which readily decomposes to urea and hydrogen peroxide.² Combining with the “night guard” bleaching technique, CP has been proven successful in providing a long-lasting tooth whitening effect.³ However, some studies have reported altered surface morphology, decreased microhardness and loss of dental hard tissue volume associated with bleaching treatments.^{4–6} Even concentrations as low as 10% CP reportedly reduce the enamel microhardness at a level comparable to that produced by higher concentrations of CP.⁷ In contrary, a study by Potocnik et al. found that 10% CP bleaching agent did not affect interior enamel microhardness but only caused local microstructural changes similar to initial caries in enamel.⁸ McCracken and Haywood reported that the amount of calcium lost from enamel exposed to 10% CP was about 1 $\mu\text{g}/\text{mm}^2$, which was not clinically significant.⁹ The literature also shows that the reduced microhardness of bleached enamel can be reversed spontaneously following a remineralization period.¹⁰ These data indicate that 10% CP is a safe self-administered bleaching agent.

Despite the proven safety of dental bleaching, patients are still concerned about potential harmful effects of these treatments such as increased tooth sensitivity. Transient tooth sensitivity during and after treatment was reported in approximately two thirds of patients undergoing bleaching.³ Conventionally, topical fluoridation is used to increase the hardness and acid resistance of demineralized teeth. Topical fluoridation has also been suggested in treating the tooth sensitivity peripherally by occluding the dentinal tubules and reducing dentinal fluid flow.¹¹ Accordingly, fluoride application is considered practicable for treating post-bleaching sensitivity.¹² The application of fluoride varnishes prior to bleaching treatment was proved to reduce dentin dehydration while the maintenance of physiological dentinal moisture can reduce hypersensitivity.¹³ As topical fluoride is applied following bleaching, mineral loss is significantly reduced.¹⁴

Recently developed bleaching agents containing additional ingredients such as fluoride, potassium nitrate and calcium phosphate have been introduced to prevent either hypersensitivity or demineralization after tooth-whitening therapy. An *in vitro* study reported that CP combined with fluoride or calcium did not prevent the reduction in surface microhardness of enamel.¹⁵ Contrarily, an X-ray photoelectron spectroscopy study revealed that additional sodium fluoride in hydrogen peroxide bleaching agent generates fluoridated hydroxyapatite and calcium fluoride crystals on enamel surfaces.¹⁶ This mechanism is believed to accelerate the remineralization process on the tooth surface. Since dental bleaching agents penetrate into dentin at certain depths to decompose intrinsic chromatic pigments,^{17,18} recent clinical evidence of associated calcium fluoride formation raises new concerns about the permeability of enamel. The travel of reactive oxygen molecules and free radicals may be impeded by crystal deposition whereas the tooth whitening effect is not comparable to conventional analogs. Based on current data, there is insufficient evaluation about the fluoridated bleaching agents.

Fluoridated bleaching agents are expected to reduce the adverse effects of tooth whitening. The influence of additional fluoride in bleaching agents on the physical properties and microstructures of enamel is still controversial and has

yet to be determined. The purpose of this study was to evaluate the effects of bleaching agents with and without fluoride as well as the post-bleaching fluoridation on bovine surface enamel. The null hypothesis was that the fluoride ingredient would not alter the whitening efficiency and demineralization effect.

2. Materials and methods

Forty non-carious bovine anterior teeth specimens were obtained from a local slaughterhouse and visually inspected to exclude those with cracks or defects. After cleansing, the specimens were stored in buffered saline until use. These bovine incisors were cut into halves using a slow speed rotary saw (Isomet 2000, Buehler, Lake Bluff, IL, USA) with water irrigation. The specimens were embedded in epoxy resin with the labial surface parallel to the horizontal plane. The enamel surfaces were then ground with 320-grit silicone carbide abrasive paper using a mechanical grinder (Ecomat 3, Buehler) to create a flat surface of approximately 5 mm \times 5 mm. The specimens were then smoothed with 600- and 1000-grit silicone carbide abrasive paper and polished with a series of 30, 9, 6 and 1 μm diamond suspension (Metadi, Buehler). The specimens were randomly divided into the following five treatment groups ($n = 16$):

- Group 1: Control specimens soaked in Hank buffer saline solution (HBSS) at 37 °C for 2 weeks. Saline solution was changed daily.
- Group 2: Specimens treated with fluoride-free bleaching agent Opalescence 10%.
- Group 3: Specimens treated as in Group 2 but with additional topical fluoridation using 2% neutral sodium fluoride gel (pH7 Gel, Pascal, Bellevue, WA, USA) for 3 min.
- Group 4: Specimens treated with a bleaching agent Opalescence PF 10% containing 0.11% fluoride.
- Group 5: Specimens treated with an experimental bleaching agent Ex-037 containing 0.37% fluoride.

Table 1 lists the bleaching agents and fluoride gel used in this study. The specimens in Groups 2–5 were bleached 8 h per day for 14 days. The bleaching agents were applied on the enamel and stored at 100% humidity at 37 °C. After each treatment, the specimens were rinsed with tap water for 1 min to remove the bleaching agent then stored in HBSS at 37 °C until the next bleaching treatment. At the end of the 14-day bleaching treatment, the specimens were stored in HBSS for an additional 14 days. Enamel changes were evaluated in three aspects: the color change, microhardness and surface topography.

2.1. Color change after bleaching treatments

Ten specimens from each group were used to determine color change and microhardness. Prior to the bleaching treatment, the color of each specimen was measured as baseline data on Day 0. The color of each specimen was assessed by the CIE-Lab system in $L^*a^*b^*$ mode using a dental colorimeter (ShadeEye NCC, Shofu, Kyoto, Japan). The samples were carefully dried

Table 1 – Bleaching agents and fluoride gel used in this study

Product	Active ingredients	pH	Manufacturer
Opalescence 10%	10% CP	6.0–6.5	Ultradent Products, Salt Lake, UT, USA
Opalescence PF 10%	10% CP, 0.11% (w/w) fluoride, 3% (w/w) potassium nitrate	6.0–6.5	Ultradent Products
Ex-037	10% CP, 0.37% (w/w) sodium fluoride	6.8	Laboratory dispensed
pH7 Gel	0.9% (w/w) sodium fluoride	7.0	Pascal, Seattle, WA, USA

but not desiccated, then placed on dark paper. The light sensor was securely positioned at right angles to the enamel surfaces. The assessed area was the cervical area, 1 mm occlusal to the cemento-enamel junction. The color of each specimen was measured three times to obtain a mean value. By using the internal software of the measurement system, color values on the $L^*a^*b^*$ color coordinate system were evaluated based upon the measured diffuse reflectance data. In this system, the “ L^* ” represents the degree of gray and corresponds to a value of brightness. The “ a^* ” is a parameter in the red-green spectrum and “ b^* ” is a parameter in the blue-yellow spectrum. After the bleaching treatment, the specimens were again inspected on Days 7 and 14 to measure the color changes ΔL^* , Δa^* , Δb^* , which indicated the increased values of lightness (L^*), and color (a^* , b^*). The overall color difference ΔE^* from the three measurements was calculated as

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

The inter-group difference was statistically analyzed by one-way analysis of variance (ANOVA) test followed by the post hoc Tukey test. Statistical significance was set at $P < 0.05$.

2.2. Microhardness test

Before the various treatments, the microhardness of the specimens used for color measurement was determined with a microhardness tester (HMV-2, Shimadzu, Kyoto, Japan). Knoop hardness (KHN) was measured with a Knoop indenter three times at a load of 50 g for 5 s. The microhardness value for each specimen prior to the treatment was set as baseline data. After 7 and 14 days, specimens in Groups 1–5, respectively, were subjected to the microhardness test. After completing the bleaching treatment, the microhardness of specimens in Groups 2–5 was continuously measured after 7 and 14 days of storage. Since Group 1 did not receive the bleaching treatment, microhardness at the recovery time was not measured. The hardness values were analyzed by the ANOVA and post hoc test for the differences among groups. Comparisons of KHN at different stages were analyzed by paired-t test.

2.3. Surface morphology observation

Three specimens from each group were examined for the surface morphology change on Day 14. All specimens were thoroughly cleaned and air-dried in a desiccator for 24 h. Following the desiccating procedure, the specimens were gold sputtered then observed under a SEM (HITACHI/S-2500, Tokyo,

Japan). Micrographs were recorded to compare the difference among these groups at magnifications of 1000 \times and 2000 \times .

3. Results

3.1. Color changes

Color change values were obtained by determining differences in L^* , a^* and b^* between Days 7, 14 and baseline (Table 2). On Day 7, the lightness change in ΔL^* was more significant than that in Δa^* and Δb^* . Group 1 showed negligible color changes for all values. The ΔL^* and overall color change ΔE^* in the bleaching groups (Groups 2–4) increased by at least 3 and 5 units, respectively.

On Day 14, both the ΔL^* and ΔE^* values in Groups 2–5 were comparable and significantly greater than those in Group 1. All color changes in Group 1 were less than 3 units, indicating a negligible whitening effect. In Groups 2–4, the color change was increased in ΔL^* and decreased in Δb^* but unchanged in Δa^* . The ΔE^* of all bleaching groups exceeded 7. Comparison of the ΔL^* , Δa^* , Δb^* values in four bleaching groups from Days 7 to 14 revealed showed significant changes.

3.2. Microhardness

The microhardness of the experimental groups from Days 0 to 28 was illustrated in Fig. 1. The baseline data were equivalent among all experimental groups ($P = 0.941$). Group 1 maintained consistent microhardness during 14 days; thus, no further microhardness measurement was performed. In each bleached group, the microhardness decreased throughout the bleaching period and gradually recovered after completion of treatment. On Day 7, Groups 2–5 showed significantly decreased enamel microhardness compared with Group 1 and respective baseline data. Group 2 showed the lowest microhardness (261.38 KHN), which significantly differed from that of Groups 1, 4 and 5. On Day 14 (completion of bleaching treatment), the microhardness in Groups 2 to 5 continued to decrease. In the bleaching groups, Group 5 demonstrated the greatest microhardness (288.95 KHN) while Group 2 had the least (238.67 KHN). Groups 2 and 3 showed significantly lower hardness value than Groups 4 and 5. On Day 21, the microhardness in the bleached groups was partially recovered. Groups 2 and 3 demonstrated similar hardness values which were significantly lower than that of Group 5. On Day 28, the hardness among four groups was not significantly different.

Paired-t test on Days 7 and 14 revealed that microhardness of the bleaching groups was significantly lower than at

Table 2 – Mean and standard error values of color differences (ΔL^* , Δa^* , Δb^* , ΔE^*) among the experimental groups throughout the bleaching period (Days 7 and 14)

	Day 7					Day 14				
	ΔL^*	Δa^*	Δb^*	ΔE^*	ΔI^*	Δa^*	b^*	ΔE^*	Δa^*	ΔE^*
Group 1: Control	1.74 ^a ± 0.84	0.10 ^a ± 0.44	0.33 ^a ± 0.40	2.52 ^a ± 1.20	2.00 ^a ± 1.51	0.43 ^a ± 0.74	0.14 ^a ± 1.30	2.64 ^a ± 1.20	0.43 ^a ± 0.74	2.64 ^a ± 1.20
Group 2: CP	4.30 ^{ab} ± 3.14	0.16 ^a ± 0.46	-0.16 ^b ± 0.39	5.26 ^{ab} ± 2.60	7.43 ^b ± 2.62	0.00 ^{ab} ± 0.30	-1.35 ^a ± 0.94	7.69 ^b ± 2.52	0.00 ^{ab} ± 0.30	7.69 ^b ± 2.52
Group 3: CP + fluoridation	3.03 ^{ab} ± 3.45	-0.37 ^{ab} ± 0.30	-0.16 ^b ± 0.22	5.68 ^b ± 2.39	5.09 ^{ab} ± 3.72	-0.53 ^{bc} ± 0.43	-3.13 ^b ± 2.36	7.13 ^b ± 1.76	-0.53 ^{bc} ± 0.43	7.13 ^b ± 1.76
Group 4: CP + 0.11%F	5.11 ^b ± 2.83	-0.73 ^b ± 0.64	-0.30 ^b ± 0.31	6.58 ^b ± 2.80	6.81 ^b ± 2.78	-1.03 ^c ± 0.63	-3.57 ^b ± 1.80	7.88 ^b ± 3.03	-1.03 ^c ± 0.63	7.88 ^b ± 3.03
Group 5: CP + 0.37%F	5.29 ^b ± 1.38	-0.47 ^{ab} ± 0.73	-0.04 ^{ab} ± 0.24	6.85 ^b ± 2.07	6.96 ^b ± 1.62	-0.43 ^{bc} ± 0.71	-4.46 ^b ± 2.55	8.49 ^b ± 2.34	-0.43 ^{bc} ± 0.71	8.49 ^b ± 2.34

Identical superscript alphabets a-c indicate no significant difference ($P \geq 0.05$) among different groups at the same stage.

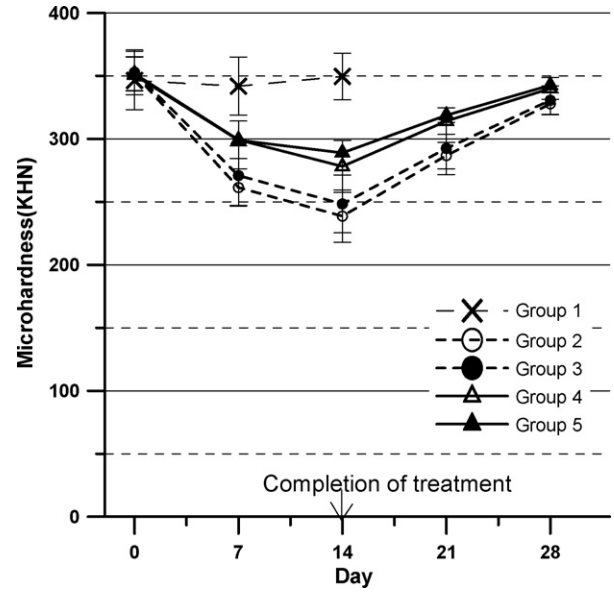


Fig. 1 – Microhardness of experimental groups during bleaching treatments and recovery time (analyzed by one-way ANOVA test).

baseline. Although the microhardness of the bleaching groups gradually recovered after bleaching, they still showed loss of hardness ranged from 8 KHN (Group 5) to 24 KHN (Group 2) on Day 28.

3.3. Surface morphology

After a 2-week treatment period, the specimens showed different surface morphology (Fig. 2). The enamel surface was unchanged on the unbleached specimen in Group 1. The surface enamel in Groups 2 and 3 showed significant alteration with erosion appearance. The erosion pattern resembled a Type II acid etching pattern, which exhibited a lost enamel prism core but retained periphery. The surface morphologic changes in Groups 4 and 5 were less distinct than those of Groups 2 and 3. They demonstrated minor dissolution of the prism core and peripheries.

4. Discussion

In the present study, enamel specimens were obtained from bovine incisors. Bovine teeth have been used to replace human teeth for years since their chemical and physical properties such as composition, hardness and tensile strength are very similar to those of human teeth.^{19,20} Despite a higher lightness with the shade of bovine enamel than in human enamel,²¹ use of young bovine teeth is considered a practicable model for evaluating bleaching methods.^{22,23} Since the present study required testing of numerous specimens, this experimental model was intended to avoid the difficulty of collecting substantial numbers of human teeth. The size of bovine teeth also facilitated the preparation of sufficient enamel surfaces for repeated microhardness tests, which enabled comparison of measured microhardness values with baseline values.

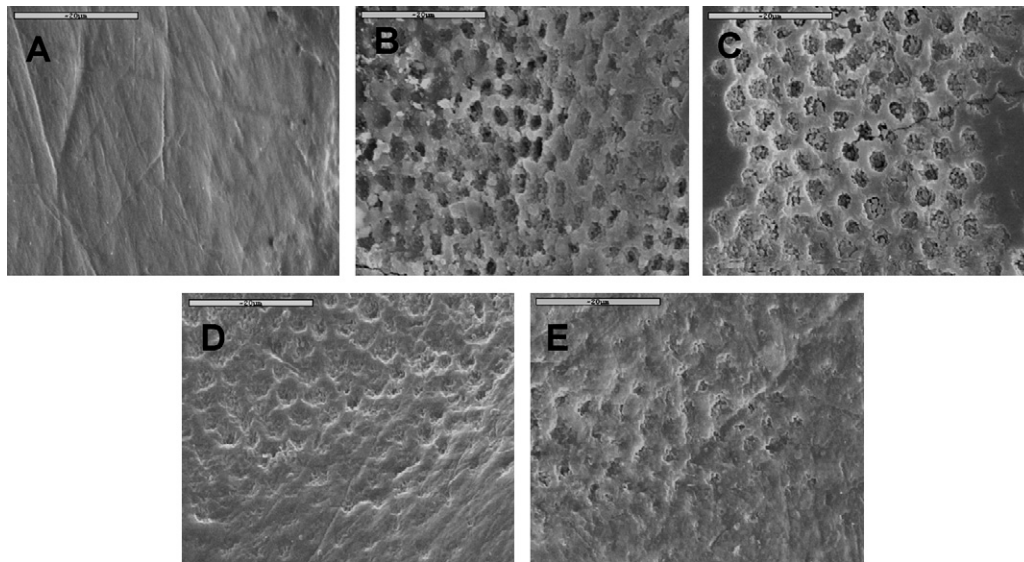


Fig. 2 – The SEM micrograph of enamel surfaces. (A) Group 1 specimen after immersion in HBSS for 2 weeks. (B) After bleaching with 10% CP for 2 weeks, the Group 2 specimen showed erosion changes with depressed enamel prism cores. (C) The Group 3 specimen showed an erosion pattern similar to that of Group 2 specimens. (D) The Group 4 specimen receiving bleaching gel containing 0.11% fluoride showed a less distinct erosion pattern with mild depressions. (E) The Group 5 specimen receiving bleaching gel containing 0.37% fluoride showed an erosion pattern similar to that of Group 4. (All in 2000 \times magnification).

Fluoride has been admitted to remineralize dental erosion lesions by increasing resistance to acid attacks by forming a calcium fluoride layer to inhibit demineralization.²⁴ The effect of post-bleaching fluoridation has also been confirmed in previous investigations. Attin et al. evaluated the remineralizing capacity of various fluoride treatments following bleaching treatment. Both fluoride varnish and solution prevented loss of hardness after bleaching.¹⁰ According to de Oliveira et al., using a desensitizing fluoride dentifrice accompanied with bleaching treatment maintained the enamel microhardness at baseline values.²⁵ In contrast with these studies, the present study evaluated the effect of fluoride on bleached enamel via approaches including post-bleaching fluoridation and fluoridated bleaching agents. Despite the widespread clinical use of fluoridated bleaching agents, their efficacy has not been thoroughly evaluated. Adding fluoride as a protective ingredient in bleaching agents may raise concerns about adverse interactions between CP and fluoride. The whitening efficiency of CP may be impaired by the calcium fluoride layer while the remineralization potential of fluoride may be hampered by CP. However, the limited available data regarding the potential problems supports the continued use of fluoridated bleaching gels. According to Wiegand et al., a fluoridated bleaching gel revealed whitening efficiency comparable to that of other nonfluoridated agents.²² A study by Burgmaier et al. showed that bovine teeth treated with CP followed by high-dosed fluoridation increased the fluoride uptake even though the structurally bound fluoride was lower than that in teeth receiving fluoridation only.²⁶ A recent study found that fluoridated bleaching gels rehardened the bleached enamel faster than nonfluoridated gels.²⁷ The teeth treated with a fluoridated bleaching agent were also reported with

higher caries resistance compared to those without bleaching treatment.²⁸ The findings of these investigations appear to support the continued use of fluoridated bleaching agents.

An earlier study showed the whitening efficiency of a fluoridated bleaching agent is equal to the fluoride-free analog using a shade guide method.²⁹ The present study used a digital colorimeter and obtained a consistent result that both fluoride-free and fluoridated bleaching agents present tooth whitening effects. This spectrophotometric/colorimetric method based on the CIE-Lab system is recognized as a reliable and objective tool for quantitative evaluation of vital tooth color change.^{30,31} In this model, changes of ΔL^* and ΔE^* values less than 1–2 units are considered as imperceptible. All the bleaching treatments altered the L^* value within 7 days. Afterward, the reduced Δb^* values indicated a decrease in yellow color saturation during Days 7 to 14. Most previous investigations confirmed that increased ΔL^* and decreased Δb^* values were expected color changes on enamel after tooth bleaching.^{22,31,32} In contrast with the minimal changes in the control group, significant increases of 5–8 units in ΔL^* and ΔE^* value were noted in the teeth receiving CP treatments, regardless of treatment protocol. These findings indicate that neither post-bleaching fluoridation nor fluoridated bleaching gel impedes the active whitening effect.

The whitening effect is achieved by decomposing CP into urea and hydrogen peroxide, which converts to perhydroxyl anion (HO_2^-) and free radicals to destroy or oxidize the double bonds in the conjugated chain of chromophore.³⁰ The available literature shows that both enamel and dentin are permeable by hydrogen peroxide and carbamide peroxide. The limited data regarding passage of active oxidizing components into dental hard tissues suggests that the peroxide can diffuse

into the enamel-dentin and even reach the pulp chamber.^{18,22,33} It is reasonable to conceive that fluoride ingredient may generate calcium fluoride deposits which alter the whitening effect and impair penetration of peroxide. However, this speculation could not be confirmed by the SEM micro-morphology in this study. The specimens treated with fluoridated bleaching agents in this study showed only minor erosive patterns and no crystal deposition on enamel surfaces. Although the presence of calcium fluoride (loosely bound fluoride) on bleached and fluoridated enamel has been reported in previous investigation,²⁶ the whitening effect of fluoridated bleaching agent was not suppressed in the present study.

Although microindentation hardness tests do not provide information about the elementary changes, they are commonly used for the material property changes in the enamel and dentin, especially following demineralization and remineralization experiments.³⁴ The variability of hardness loss in different studies was contributed to factors including the test method, the demineralization degree of the bleached enamel, and the immersion media. According to Potocnik et al., the demineralization by a 10% CP bleaching agent did not reduce microhardness in a Vickers hardness test.⁸ However, a Knoop indenter penetrates the enamel with low depth.²⁶ Considering the demineralization change is limited to surface enamel, a Knoop hardness test is more suitable than a Vickers hardness test to differentiate the hardness change. Previous investigations reported 12–40% microhardness losses on 10% CP treated enamel using a Knoop hardness test.^{35,36} In the present study, the initial microhardness of all groups ranged from 340 to 352 KHN. The decreased microhardness in Groups 2–5 after 14-day bleaching equals to a 18–32% reduction in microhardness, which is in accord with the previous Knoop hardness tests. The degree of demineralization change caused by the bleaching agents was related to their concentration and the pH value after buffering with other additives. A recent study found that some consumer-available, over-the-counter bleaching solutions caused more adverse microhardness change than 10% CP.³⁷ 10% CP is considered as a relatively safe bleaching agent with its limited demineralization effect.

In the experiment, the specimens were immersed in HBSS instead of artificial saliva for 16 h between the applications. Saliva is a natural remineralization source for demineralized enamel. Shannon et al. demonstrated *in vivo* remineralization of bleached enamel exposed to natural saliva.⁴ Artificial saliva has also proven effective for rehardening softened enamel surfaces.³⁸ Compared to artificial saliva, HBSS exhibited more electrolytes and higher calcium ion concentration (2.6 mM). Accordingly, the automatic reversal of hardness values in Group 2 after Day 28 may have been attributable to the accelerated remineralizing ability of HBSS. However, the microhardness of the bleached groups did not return to baseline values after immersion in HBSS for 2 weeks. Change in hardness might reflect a change in the surface layers of tooth structure such as porosity and erosion of enamel and dentin. Remineralization may not always be complete, leaving areas susceptible to further decalcification and plaque retention.

During the first 14 days, the microhardness of the control group specimens was significantly higher than that in other groups regardless of fluoridation protocol. The fluoridated

bleaching agents demonstrated greater resistance to erosion and increased reversed microhardness than groups treated with only bleaching and fluoridation. The 10% CP bleaching gel caused moderate demineralization on enamel surfaces. However, bovine teeth in this study did not receive regular toothpaste fluoridation. The demineralization in pre-fluoridated human enamel may be less aggressive. Although previous studies show that post-treatment fluoridation increases the resistance of enamel to erosive attacks and recovers the microhardness,^{10,39} it proved inferior to intrinsic fluoride in this study. As described in previous studies, the opening of diffusion channels on demineralized enamel facilitates diffusion of fluoride into deeper enamel layers and enhances remineralization.⁴⁰ In the current study, a milder etched change on enamel treated with fluoridated bleaching gels was also consistent with this speculation. Previous studies have shown that post-bleaching fluoridation effectively prevents mineral loss.^{14,25} This study showed that fluoridated bleaching gels preserve enamel hardness more effectively than fluoridation during bleaching treatment and the recovery stage. The irrelevant mineral preservation with post-bleaching fluoridation was associated with the low concentration of fluoride. A recent study proved the remineralizing ability of a high concentration of fluoride gel to restore enamel hardness near a level of non-bleached enamel.³⁶ It should be noticed that the bleaching and post-fluoridation groups may take more than 2 weeks to regain their original hardness from the immersed saliva after completion of the bleaching therapy. These erosive lesions are susceptible a consequence of acid attacks and abrasion such as tooth brushing *in vivo* which may lead to irreversible surface loss.⁴¹ Although any demineralization change in bleached enamel may be reversed by salivary electrolytes, a milder demineralization change and short recovery time helps to prevent the erosion-abrasion lesion formation. Therefore, the fluoride supplement either during or after bleaching is considered beneficial for preventing enamel demineralization.

The Ex-037 used in Group 5 contained a higher fluoride concentration (0.37%, 1665 ppm) than that in the commercial formula (10% Opalescence PF). The fluoride concentration was close to that required in European cosmetic toothpaste (1500 ppm) and no higher than that in some fluoride varnishes⁴²; thus, it complies with current regulations. Groups 4 and 5 did not significantly differ in loss of hardness during the 2-week treatment. According to Attin et al., a bleaching gel containing 0.5% fluoride causes insignificant hardness loss in spite of the shorter bleaching period required.²⁷ Higher fluoride concentration would be expected to enhance erosion inhibition ability, especially after longer bleaching periods. However, further investigation is needed to clarify the efficiency and safety of the new formula before it can be applied clinically.

5. Conclusions

The present study examined the effect of fluoridated bleaching agents and post-bleaching fluoridation on bovine enamel surface characteristics. The enamel microhardness was found to decrease for all bleaching treatments but recovered

gradually after completion of bleaching. In all bleaching groups, whitening efficiency was similar on the aspects of increase in whiteness and decrease in yellow color saturation. Despite the limitations of this *in vitro* study, the experimental results suggest that fluoridated bleaching gel produces less demineralization changes such as the erosion morphology and hardness loss without compromising whitening efficiency. However, further studies are required to evaluate the clinical effects of the fluoridated bleaching gel as well as the optimal fluoride concentration for maximum tooth whitening and minimum side-effects.

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REFERENCES

- Christensen GJ. Bleaching teeth: practitioner trends. *Journal of the American Dental Association* 1997;128:16S-8S.
- Haywood VB, Heymann HO. Nightguard vital bleaching: how safe is it? *Quintessence International* 1991;22:515-23.
- Haywood VB, Leonard RH, Nelson CF, Brunson WD. Effectiveness, side effects and long-term status of nightguard vital bleaching. *Journal of the American Dental Association* 1994;125:1219-26.
- Shannon H, Spencer P, Gross K, Tira D. Characterization of enamel exposed to 10% carbamide peroxide bleaching agents. *Quintessence International* 1993;24:39-44.
- Wandera A, Feigal RJ, Douglas WH, Pintado MR. Home-use tooth bleaching agents: an *in vitro* study on quantitative effects on enamel, dentin, and cementum. *Quintessence International* 1994;25:541-6.
- Ben-Amar A, Liberman R, Gorfil C, Bernstein Y. Effect of mouthguard bleaching on enamel surface. *American Journal of Dentistry* 1995;8:29-32.
- Faraoni-Romano JJ, Turssi CP, Serra MC. Concentration-dependent effect of bleaching agents on microhardness and roughness of enamel and dentin. *American Journal of Dentistry* 2007;20:31-4.
- Potocnik I, Kosec L, Gaspersic D. Effect of 10% carbamide peroxide bleaching gel on enamel microhardness, microstructure, and mineral content. *Journal of Endodontics* 2000;26:203-6.
- McCracken MS, Haywood VB. Demineralization effects of 10 percent carbamide peroxide. *Journal of Dentistry* 1996;24:395-8.
- Attin T, Kielbassa AM, Schwanenber M, Hellwig E. Effect of fluoride treatment on remineralization of bleached enamel. *Journal of Oral Rehabilitation* 1997;24:282-6.
- Paes Leme AF, dos Santos JC, Giannini M, Wada RS. Occlusion of dentin tubules by desensitizing agents. *American Journal of Dentistry* 2004;17:368-72.
- Haywood VB. Current status of nightguard vital bleaching. *Compendium of Continuing Education in Dentistry* 2000;S10-7.
- Betke H, Kahler E, Reitz A, Hartmann G, Lennon A, Attin T. Influence of bleaching agents and desensitizing varnishes on the water content of dentin. *Operative Dentistry* 2006;31:536-42.
- Bizhang M, Seemann R, Duve G, Romhild G, Altenburger JM, Jahn KR, et al. Demineralization effects of 2 bleaching procedures on enamel surfaces with and without post-treatment fluoride application. *Operative Dentistry* 2006;31:705-9.
- de Oliveira R, Paes Leme AF, Giannini M. Effect of a carbamide peroxide bleaching gel containing calcium or fluoride on human enamel surface microhardness. *Brazilian Dental Journal* 2005;16:103-6.
- Tanizawa Y. Reaction characteristics of a tooth-bleaching agent containing H₂O₂ and NaF: *in vitro* study of crystal structure change in treated hydroxyapatite and chemical states of incorporated fluorine. *Journal of Cosmetic Science* 2005;56:121-34.
- Gokay O, Tuncbilek M, Ertan R. Penetration of the pulp chamber by carbamide peroxide bleaching agents on teeth restored with a composite resin. *Journal of Oral Rehabilitation* 2000;27:428-31.
- Suliman M, Addy M, Macdonald E, Rees JS. The bleaching depth of a 35% hydrogen peroxide based in-office product: a study *in vitro*. *Journal of Dentistry* 2005;33:33-40.
- Urabe I, Nakajima S, Sano H, Tagami J. Physical properties of the dentin-enamel junction region. *American Journal of Dentistry* 2000;13:129-35.
- Mellberg JR. Hard-tissue substrates for evaluation of cariogenic and anti-cariogenic activity *in situ*. *Journal of Dental Research* 1992;71:913-9.
- Kwon YH, Huo MS, Kim KH, Kim SK, Kim YJ. Effects of hydrogen peroxide on the light reflectance and morphology of bovine enamel. *Journal of Oral Rehabilitation* 2002;29:473-7.
- Wiegand A, Vollmer D, Foitzik M, Attin R, Attin T. Efficacy of different whitening modalities on bovine enamel and dentin. *Clinical Oral Investigations* 2005;9:91-7.
- Attin T, Vollmer D, Wiegand A, Attin R, Betke H. Subsurface microhardness of enamel and dentin after different external bleaching procedures. *American Journal of Dentistry* 2005;18:8-12.
- White DJ, Featherstone JD. A longitudinal microhardness analysis of fluoride dentifrice effects on lesion progression *in vitro*. *Caries Research* 1987;21:502-12.
- de Oliveira R, Basting RT, Rodrigues JA, Rodrigues Jr AL, Serra MC. Effects of a carbamide peroxide agent and desensitizing dentifrices on enamel microhardness. *American Journal of Dentistry* 2003;16:42-6.
- Burgmaier GM, Schulze IM, Attin T. Fluoride uptake and development of artificial erosions in bleached and fluoridated enamel *in vitro*. *Journal of Oral Rehabilitation* 2002;29:799-804.
- Attin T, Betke H, Schippan F, Wiegand A. Potential of fluoridated carbamide peroxide gels to support post-bleaching enamel re-hardening. *Journal of Dentistry* 2007;35:755-9.
- Al-Qunaian TA. The effect of whitening agents on caries susceptibility of human enamel. *Operative Dentistry* 2005;30:265-70.
- Gladwell J, Simmons D, Wright JT. Remineralization potential of a fluoridated carbamide peroxide whitening gel. *Journal of Esthetic and Restorative Dentistry* 2006;18:206-12.
- Joiner A. The bleaching of teeth: a review of the literature. *Journal of Dentistry* 2006;34:412-9.
- Dietschi D, Rossier S, Krejci I. *In vitro* colorimetric evaluation of the efficacy of various bleaching methods and products. *Quintessence International* 2006;37:515-26.
- Kugel G, Petkevis J, Gurgan S, Doherty E. Separate whitening effects on enamel and dentin after fourteen days. *Journal of Endodontics* 2007;33:34-7.

33. Thitinanthapan W, Satamanont P, Vongsavan N. In vitro penetration of the pulp chamber by three brands of carbamide peroxide. *Journal of Esthetic Dentistry* 1999;11: 259–64.
34. Featherstone JD, ten Cate JM, Shariati M, Arends J. Comparison of artificial caries-like lesions by quantitative microradiography and microhardness profiles. *Caries Research* 1983;17:385–91.
35. Zantner C, Beheim-Schwarzbach N, Neumann K, Kielbassa AM. Surface microhardness of enamel after different home bleaching procedures. *Dental Materials* 2007;23: 243–50.
36. da Costa JB, Mazur RF. Effects of new formulas of bleaching gel and fluoride application on enamel microhardness: an in vitro study. *Operative Dentistry* 2007;32:589–94.
37. Leonard RH, Teixeira EC, Garland GE, Ritter AV. Effect on enamel microhardness of two consumer-available bleaching solutions when compared with a dentist-prescribed, home-applied bleaching solution and a control. *Journal of Esthetic and Restorative Dentistry* 2005;17:343–50.
38. Attin T, Buchalla W, Gollner M, Hellwig E. Use of variable remineralization periods to improve the abrasion resistance of previously eroded enamel. *Caries Research* 2000;34:48–52.
39. Lewinstein I, Fuhrer N, Churaru N, Cardash H. Effect of different peroxide bleaching regimens and subsequent fluoridation on the hardness of human enamel and dentin. *The Journal of Prosthetic Dentistry* 2004;92:337–42.
40. Klimek J, Hellwig E, Ahrens G. Fluoride taken up by plaque, by the underlying enamel and by clean enamel from three fluoride compounds in vitro. *Caries Research* 1982;16:156–61.
41. Vieira A, Lugtenborg M, Ruben JL, Huysmans MC. Brushing abrasion of eroded bovine enamel pretreated with topical fluorides. *Caries Research* 2006;40:224–30.
42. Bloch-Zupan A. Is the fluoride concentration limit of 1,500 ppm in cosmetics (EU guideline) still up-to-date? *Caries Research* 2001;35:22–5.