Analysis of Tooth Enamel After Excessive Bleaching: A Study Using Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy

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This study assessed alterations on bovine enamel after excessive bleaching. Coronal portions of bovine teeth (n = 30) were sectioned and divided into three groups (n = 10 per group). The coronal parts were further cut incisocervically into two halves. While one half received no bleaching (control), the other half was subjected to either one (group 1), three (group 2), or five bleaching sessions (group 3) with 35% hydrogen peroxide. The enamel surfaces were then analyzed using scanning electron microscopy and energy dispersive x-ray spectroscopy (EDS). Excessive bleaching affected the surface morphology and chemistry of the bovine enamel. EDS analysis showed the highest decrease in calcium ion percentages in groups 2 and 3 when compared to their nonbleached halves. Oxygen and phosphorus percentages were comparable on both the control and bleached enamel, regardless of the number of bleaching sessions. Consecutive bleaching sessions with 35% hydrogen peroxide may lead to morphologic and specific elemental changes when performed in a short period of time. Calcium ion percentages may decrease when this bleaching agent is used for more than one session. *Int J Prosthodontics 2010;23:29–32.*

Dental bleaching using peroxides is considered to be a conservative treatment approach with satisfactory results in the majority of clinical cases where patients present with one or more discolored teeth. However, there are some reports demonstrating the negative effects of such peroxides on hard[1] and soft tissues[2] in the oral cavity. Nevertheless, in an attempt to achieve whiter teeth in a short time span, many professionals tend to use bleaching agents repeatedly, without concern for the possible structural changes on the hard dental tissues during or after bleaching.

This study assessed the morphologic and elemental composition of enamel surfaces after the repeated use of a high-concentration bleaching agent in a short period of time. The null hypothesis tested was that excessive bleaching would not affect the enamel structure microscopically.

Materials and Methods

Production of Specimens

Bovine mandibular incisors (n = 30) were collected and the coronal parts sectioned. The enamel surfaces were cleaned and polished using water and fluoride-free pumice (3M ESPE) with a prophylaxis brush for 15 minutes at a slow speed. Subsequently, specimens were ultrasonically cleaned (Vitasonic II, Vita) in distilled water for 15 minutes and air dried. In the middle third

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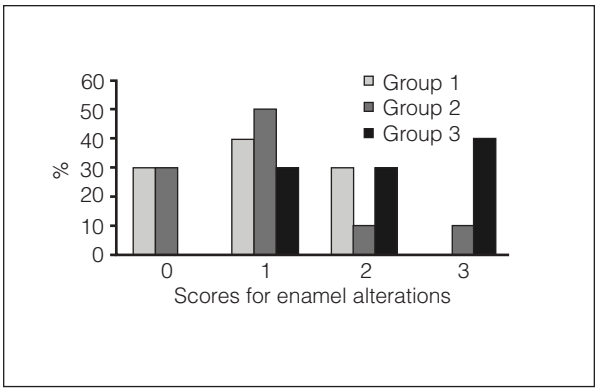


Fig 1 Distribution of scores for the enamel alterations given after SEM analysis. 0 = enamel surface without alterations, 1 = enamel surface with superficial porosities covering less than 30% of the area, 2 = enamel surface with superficial porosities covering between 30% and 60% of the area, and 3 = enamel surface with superficial porosities covering more than 60% of the area.

of the labial surface of each tooth, a circular adhesive tape with a 5-mm-diameter opening was attached. Using double-sided diamond disks (KG Sorensen), each tooth was cut incisocervically into two halves and square-shaped enamel blocks were obtained 1 mm away from the margins. While one half received no bleaching agent (control), the other half was subjected to one (group 1), three (group 2), or five bleaching sessions (group 3) with 35% hydrogen peroxide (Whiteness HP 35%, FGM) (n = 10 per group). The bleaching agent was applied with the aid of a microbrush. One bleaching session consisted of three applications of the gel for 15 minutes each, yielding to 45 minutes total per session (group 1: 45 minutes, group 2: 135 minutes, group 3: 225 minutes).

The experimental specimens were stored in artificial saliva (pH = 6, 37 ± 1ºC; Biofórmula) for 2 days between each bleaching session. Specimens in the control group not receiving any bleaching agent were also stored in artificial saliva.

SEM Analysis

After bleaching, specimens were analyzed using scanning electron microscopy (SEM) (JSM-5400, Jeol) (magnification ×1,000 and ×2,000). The following scores were given depending on the size of the alterations to the enamel surface: 0 = enamel surface without alteration, 1 = enamel surface with superficial porosities covering less than 30% of the area, 2 = enamel surface with superficial porosities covering between 30% and 60% of the area, and 3 = enamel surface with superficial porosities covering more than 60% of the area.

EDS Analysis

To determine the elements present on the enamel both before and after bleaching, energy dispersive x-ray spectroscopy (EDS) (SEM JEOL-JSM-5400, Jeol, equipped with INCA Energy Program, Oxford Instruments) was performed on a separate set of teeth (n = 2 per group). In each measurement area (1 mm^2), the intensity profile of the major elements present was analyzed. The accelerating voltage was set at 15 kV with a working distance of 15 mm. The x-ray detector was set at 5 cm throughout the experiment under secondary electron (SE) mode at a magnification of ×800. The spectra and net intensity of the detected elements for each spot was collected at approximately 4,000 counts in 100 seconds. Concentrations were determined after calculating the average percentage of the weight of a particular element at each spot (dual time: 20% to 30% saturation).

Statistical Analysis

The scores obtained from both the control and experimental groups were statistically analyzed using Statistix for Windows (version 8.0, Analytical Software) and the Kruskal-Wallis one-way and Dunn (10%) tests. *P* values less than .05 were considered statistically significant in all tests.

Results

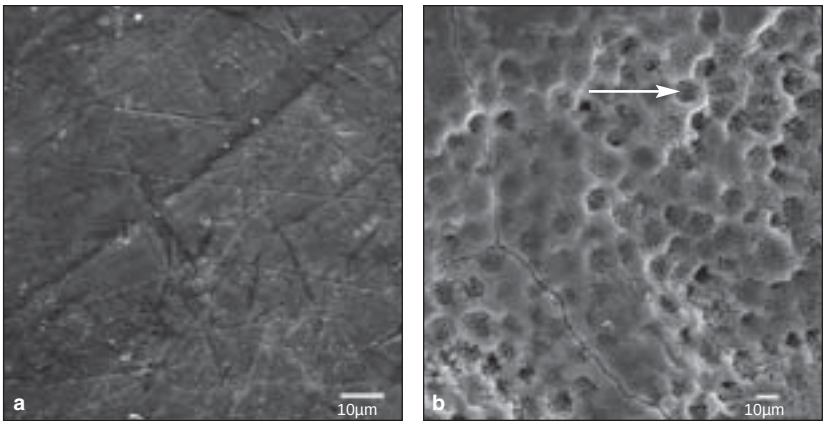
Excessive bleaching had significant effects on the bovine enamel of the experimental groups when compared to the control group ($P = .020$). Group 3 presented a significantly higher incidence of score 3 ($P < .05$) during SEM analysis than groups 1 and 2 (Fig 1). Therefore, the null hypothesis was rejected.

Surface alterations in bovine enamel after bleaching regimens before and after five sessions of bleaching are displayed in Figs 2a and 2b.

EDS analysis showed the highest decrease in calcium ion percentages in groups 2 and 3 when compared to their nonbleached halves (Table 1). Percentages of oxygen and phosphorus were comparable between both halves in the control and the bleached groups.

Discussion

Bleaching agents with lower concentrations clinically perform within safety margins. The purpose of this study, however, was to investigate the extreme effects of bleaching agents on bovine enamel, especially in cases of severe discoloration or patients undergoing secondary bleaching after a relapse when bleaching



Figs 2a and 2b Representative SEM micrographs of bovine enamel for one tooth in group 3 from **(a)** the nonbleached control half (score: 0, magnification ×1,000) and **(b)** the bleached half of the same tooth after five sessions of bleaching (score: 3, magnification ×2,000). Note the enamel surface with many exposed enamel prisms *(arrow)*.

Table 1 EDS Analysis Regarding the Elemental Composition of the Enamel Surface

	Quantity (%)					
	Group 1		Group 2		Group 3	
Element	A	B	A	B	A	B
Oxygen	45.11	45.61	45.95	47.58	46.20	47.92
Calcium	26.68	25.71	34.73	29.01	32.06	26.77
Phosphorus	12.21	12.26	12.03	14.02	12.75	13.83

A = unbleached half; B = bleached half.

was not performed cautiously. In previous studies, bovine enamel has been reported to be a comparable substrate to human enamel.[3,4]

SEM analysis of the bovine enamel surfaces showed increased surface alterations directly proportional to the period of exposure to the bleaching agent. In an earlier study by Hosoya et al,[5] an increase in surface roughness in human enamel was noted with each additional application of 35% carbamide peroxide. Bleaching agents penetrate the tooth structure, denature the proteins, increase the permeability of the tissues, and allow the passage of hydrogen ions to the tooth, causing alterations in the dental hard tissues.[1,5] According to the EDS analysis, a reduction in calcium ion levels was observed after bleaching three and five times, demonstrating the demineralizing effect of this bleaching agent. Similar effects were observed on enamel, dentin, and cement structures using 30% hydrogen peroxide or 10% carbamide peroxide at varying degrees.[6] The results of this study are of course only valid for the bleaching agent used. Further studies with other bleaching agents, while also considering the pH effect of the environment, are required, preferably in vivo.

Although visible color changes were evident at the end of each bleaching session, no attempt was made to verify the color of the enamel using a colorimeter or other color-measuring device. The interest of this study was solely on the physical and chemical structural changes of the enamel. In clinical situations, it may be possible that when the color change is not sufficient, bleaching sessions may be prolonged, resulting in longer sessions than studied in this investigation. This decision is often dictated by the desired level of color change, which may lead to further deterioration than observed in this study.

Although it was not the main purpose of this investigation, for lengthy bleaching applications, the original structure of the enamel can be restored through regular toothpaste fluoridation. This approach was reported to prevent loss of microhardness due to bleaching treatment in vitro more than an additional supplementation of a fluoride gel.[7] However, controversial results exist on this topic since no supporting evidence regarding the influence of fluoride-containing bleaching gels on remineralization has been documented.[8] As an alternative, the use of fluoridated bleaching agents that produce less demineralization of the surface morphology and microhardness are advised.[9] However, this needs to be verified in future investigations.

Conclusion

The indiscriminate and overuse of the 35% hydrogen peroxide–based bleaching agent tested in this study has the potential to cause morphologic and chemical alterations to the enamel structure.

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Literature Abstract

The effects of smoking on the survival of smooth- and rough-surface dental implants

The objective of this retrospective clinical study was to compare the long-term survival rates of smooth- and rough-surface dental implants placed in smokers and nonsmokers in a single institution. A retrospective chart review was completed for all consenting patients treated with endosseous dental implants at the Mayo Clinic, Rochester, Minnesota, between two time periods. The first time period of January 1, 1991 through December 31, 1996 was chosen to report clinical performance of Nobel Biocare Brånemark smooth-surface implants. The second time interval between January 1, 2001 and December 31, 2005, was to reflect on the clinical performance of Nobel Biocare rough-surface implants, which utilize the Ti-Unite surface. Implants for the first and second time periods were followed through mid-1998 and mid-2007, respectively. The duration of follow-up for each implant was calculated from the time of placement to the date of failure or date of last follow-up in the specified time period. Implant survival was estimated using Kaplan-Meier statistics. Associations between implant survival and patient and implant variables were investigated with marginal Cox proportional hazards models (adjusted for age and sex), and summarized by hazard ratios (HR) and corresponding 95% confidence intervals (CI). A total of 593 patients (322 females, 271 males; mean age: 51.3 years) received 2,182 smooth-surface implants from 1991 to 1996. One hundred four (17.5%) patients in this group were smokers. Nine hundred five patients (539 females, 366 males; mean age: 48.2 years) were studied between 2001 and 2005. This group, of which 95 patients (10.5%) were smokers, received 2,425 rough-surface implants. Among the rough implants, smoking was not significantly associated with implant failure (HR = 0.8; 95% CI = 0.3–2.1; $P = .68$). Smoking was significantly associated with implant failure in the smooth implant group (HR = 3.1; 95% CI = 1.6–5.9; $P < .001$) and smooth implants were 3.1 times more likely to fail than rough implants in smokers (95% CI = 1.1–9.0; $P = .039$). Anatomical location was not significantly associated with implant survival in both smoker and nonsmoker patients with rough-surface implants ($P = .45$). For smooth implants, anatomical location significantly affected survival rates in smokers ($P = .004$) but not nonsmokers ($P = .17$). Implant survival was the poorest for smooth implants placed in the posterior maxillae of smokers (65.3% survival at 5 years). The authors identified several factors, such as systemic conditions of the patients, reasons of tooth loss, and whether grafting or membranes were used, that were not considered and thus could be a disadvantage in this study. Nevertheless, given the steady decrease of survival rates of smooth-surface implants in smokers, it may be prudent to place these patients on a strict recall regimen so that impending failure of those implants can be detected early.

Balshe AA, Eckert SE, Koka S, Assad DA, Weaver AL. *Int J Oral Maxillofac Implants* 2008;23:1117–1122. **References:** 34. **Reprints:** Dr Ayman A. Balshe. Email: abalshe@hotmail.com—*Elvin W.J. Leong, Singapore*