INTERNATIONAL JOURNAL OF IMMUNOPATHOLOGY AND PHARMACOLOGY Vol. 25, no. 4, 1021-1027 (2012)

HISTOMORPHOLOGIC ALTERATIONS OF HUMAN ENAMEL AFTER REPEATED APPLICATIONS OF A BLEACHING AGENT

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Received May 7, 2012 - Accepted July 31, 2012

The objective of the study was to analyse the histomorphology of enamel after repeated applications of a professional bleaching regimen. Enamel specimens were obtained from buccal surfaces of 20 extracted human incisors. Two specimens were obtained for each tooth. Half of each tooth was maintained in distilled water and served as control; the other part was treated with a 38% hydrogen peroxide professional bleaching agent. The treated specimens were divided in four groups: in group 1, the professional bleaching protocol suggested by the manufacturer was applied once; in group 2, the same protocol was repeated twice; in group 3, three times; in group 4, four times. Between bleaching applications and before SEM evaluation, enamel specimens were maintained in artificial saliva for 1 week. Enamel specimens for each group were submitted to a qualitative scanning electron microscopy (SEM) analysis (200X, 500X, 1000X, 3000X), comparing the treated specimens with the related control enamel. Results of the SEM analysis showed no relevant alteration on the enamel surfaces, when the bleaching protocol was applied once or twice. However, significant changes of enamel surface morphology were SEM observed in groups 3 and 4, suggesting a predominance of depressions when bleaching procedure was repeated three or four times. From the results of this *in vitro* study, it is possible to state that bleaching procedures should not be carried out indiscriminately. SEM analysis showed important alterations of the prismatic structure of the enamel when the bleaching protocol was applied three and four times.

Since the introduction of the tooth-whitening technique that uses custom bleaching trays loaded with 10 percent carbamide peroxide gel in 1989 (1), the demand for information on tooth bleaching and whitening has increased dramatically. In the last years, tooth bleaching has become popular in dentistry since it has been shown to be a conservative alternative to direct or indirect restoration of either stained or darkened teeth (2).

Although exogenous bleaching has been long

Key words: enamel, hydrogen peroxide, scanning electron microscopy, tooth whitening

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0394-6320 (2012)

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

used and widely accepted as a clinical procedure, little is understood about its interaction with hard and soft tissues of the oral cavity, besides the possible consequences of these reactions in the long term (3, 4). Diverging results have been published with respect to the consequences of the influence of whitening treatments on the alteration of enamel surface. Although some authors have pointed out that tooth bleaching with selected commercial H_2O_2 or carbamide peroxide do not produce modifications in surface morphology (5-7), another group of reports found that bleaching agents create some enamel porosity (8, 9). For example, Ruse et al. (10) have shown that in bleached enamel, the calcium/phosphate ratio is altered by a 35% H_2O_2 treatment. According to Seghi and Denry (11), enamel treated with a 10% (w/w) carbamide peroxide gel displays a reduction in apparent fracture toughness (ca. 30%), with no significant changes in surface hardness. Data acquired indicate small but significant decreases in abrasion resistance. To explain these findings, the authors hypothesized that the chemical action of H_2O_2 induced an alteration of the organic matrix of enamel.

Differences between these published reports may arise from a variation in the protocols, the chemicals that were tested and their concentrations, as well as the methods used to evaluate the effects (12). In recent years a "tooth whitening mania" has increased and some patients who are not satisfied with the results of a single bleaching ask for repeated sessions of professional tooth whitening to obtain the ideal smile that they have in mind (13).

The aim of this study was to assess the histomorphology of the enamel surface of human teeth, after repeated applications of a professional bleaching regimen based on the use of hydrogen peroxide at high concentration.

MATERIALS AND METHODS

Twenty freshly extracted under orthodontic indication single-rooted human teeth were selected (patients mean age: 19 years; patients range: 15-31 years) and stored in 0.5% chloramine-T aqueous solution at 4°C until the beginning of experiment, but no longer than 1 week after extraction. Teeth with defects or cracks were excluded. The roots of these teeth were amputated 2 mm apically at the buccal cement-enamel junction using a cylindrical diamond rotary cutting instrument (Intensiv 314, Ø ISO 014, L.8.0 mm; Intensiv, Grancia, Switzerland) mounted on a high-speed handpiece (Bora L; Bien-Air, Bienne, Switzerland) with water-spray cooling. The coronary remnant was polished with pumice and brush in a contra-angle (Intramatic; Kavo, Germany). The anatomic crowns were then bucco-lingually sectioned into two halves, with a low speed diamond saw (Micromet M; Remet S.p.A., Casalecchio di Reno, Italy), under water lubrication.

The specimens were divided in four groups. Half part of each tooth was maintained in distilled water and served as control; the other part was treated with a 38% hydrogen peroxide professional bleaching agent (Opalescence Boost; Ultradent Products, Inc., South Jordan, Utah, USA) (Table I). For the gel application protocol, the manufacturer's instructions were followed. In group 1, a professional whitening session was a simulated, with three applications of gel, each of which lasted 15 minutes. Between applications, the surfaces of specimens were rinsed under tap water for about a minute and then air-dried. In group 2, the same protocol was repeated twice; in group 3, three times; in group 4, four times. Between active periods of bleaching protocols and before SEM evaluation, enamel specimens were maintained in artificial saliva (KCl 50 mmol/L, Ca^{2+} 1.5 mmol/L, PO_4^{3-} 0.9 mmol/L, trihydroxymethylamino-methane buffer 20 mmol/L, at pH 7.0) for 1 week.

Enamel specimens for each group were then submitted to a qualitative scanning electron microscopy (SEM) (XL30CP; Philips) analysis (200X, 500X, 1000X, 3000X), comparing the treated specimens with the related control enamel. SEM images, viewed on a monitor, were evaluated by two examiners and scored as follows: (a) smooth, normal enamel; (b) fissures on the enamel surface; (c) images of mildly increased porosity; (d) images of exposed enamel prisms and dissolution aspects.

RESULTS

Results of the SEM analysis showed no relevant alterations respect to controls on the enamel surfaces, when the bleaching protocol was applied once or twice (Figs. 1, 2). The surface of untreated enamel was rarely completely smooth, however the aprismatic surface layer was always uniform. In some specimens from groups 1 and 2, as well as from control group, some pores and superficial irregularities, such as grooves were observed. In such a case, specimens were labeled with score "b". In two cases from group 2, a mildly increased porosity was detected. It consisted in areas of depressions

Table I. *Details about bleaching agent used: manufacturer, concentration, pH, number of applications and total tooth contact time.*

Bleaching system	Batch n.	Bleaching material dispensing	H_2O_2 %	pH	Number of application x time / cycle	Total contact time / cycle
Opalescence Boost Ultradent Products, Inc., South Jordan, Utah, USA.	5330-B4S9N	Gel supplied in 2 separate syringes Mixed and applied with mixing tip	38%	7	3 x 15 min / cycle	45 min / cycle

that seemed sometimes deeper, generating a more variable aspect of the enamel surface. In these cases, specimens were labelled with score "c" (Table II).

On the other hand, significant changes of enamel surface morphology were SEM observed in groups 3 and 4, suggesting a predominance of depressions when bleaching procedure was repeated three or four times (Figs. 3, 4). All the specimens from groups 3 and 4 were labelled with score "c" or "d" (Table II).

DISCUSSION

The efficacy of whitening products is well documented but the effects of their application on enamel surface is poorly understood with contradictory evidence from in vitro studies (3). The present study raises the question about bleaching potential effects on the enamel surface integrity. The bleaching procedure adopted in the current study simulated an in-office bleaching procedure, using a 38% hydrogen peroxide professional bleaching agent. The bleaching cycle was repeated up to four times to evaluate the effects on enamel surface of repeated application of this highly concentrated whitening solution.

The results of the study showed no considerable differences on the histomorphology of the enamel surface compared to the control specimens when the bleaching protocol was applied once or twice, as displayed in Figs. 1 and 2. Minor irregularities of the enamel surface occurred in specimens treated with one or two application cycles of Opalescence Boost, respectively with 45 or 90 minutes of total contact time. These irregularities are difficult to be considered as secondary effects of the treatment. In fact, when the enamel surfaces were examined in the control group, pores, shallow depressions and superficial irregularities were often observed, as previously reported by other authors (14, 15). On the surface of normal, sound teeth, circumferentially horizontal lines may be found across the face of the crown; moreover, lamellae or cracks are not unusual. Previous SEM observations showed that enamel surface varies with age and that patient's diet or oral hygiene habits might be responsible of these surface irregularities (15, 16).

Table II. *Scores of SEM images for enamel specimens of each group.*

Groups	Number of specimens labeled for each score *			
	a	*b*	*c*	*d*
Control Group (No bleaching)	27	13	-	-
Group 1 (1 bleaching cycle)	6	4	-	-
Group 2 (2 bleaching cycles)	5	3	2	-
Group 3 (3 bleaching cycles)	-	-	7	3
Group 4 (4 bleaching cycles)	-	-	5	5

** **(a)** smooth, normal enamel; **(b)** fissures on the enamel surface; **(c)** images of mildly increased porosity; **(d)** images of exposed enamel prisms and dissolution aspects.*

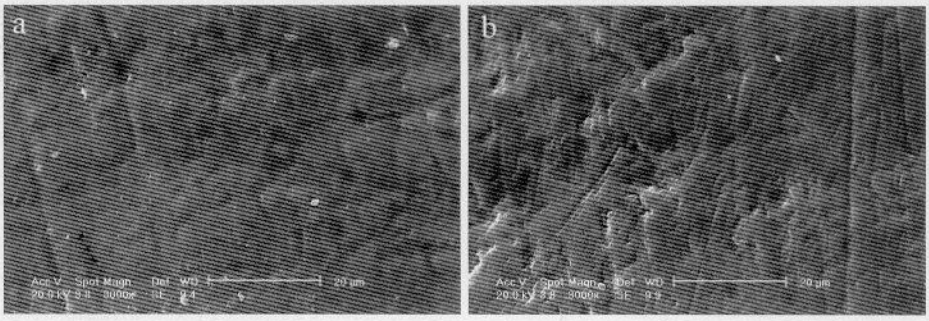

Fig 1. *Control **(a)** and experimental **(b)** enamel from group 1. The surface of untreated enamel (a) appears completely smooth. On the treated enamel (b) some pores and lines are evident.*

The major finding of this study was that moderate to severe enamel surface modifications were found when the bleaching protocol was repeated three or four times, as displayed in Figs. 3 and 4. A predominance of depressions was SEM visualized, suggesting important alterations of the prismatic structure of the enamel. Focal areas of shallow erosion, loss of the prismatic layer, pitting, and exposure of the enamel prisms were often observed. Such undesirable effects may have some implication on future developments regarding the carious decay (3). In fact, changes in surface roughness could influence the formation of supra- and sub-gingival plaque (17). However, it should be recognized that up to now, there are no

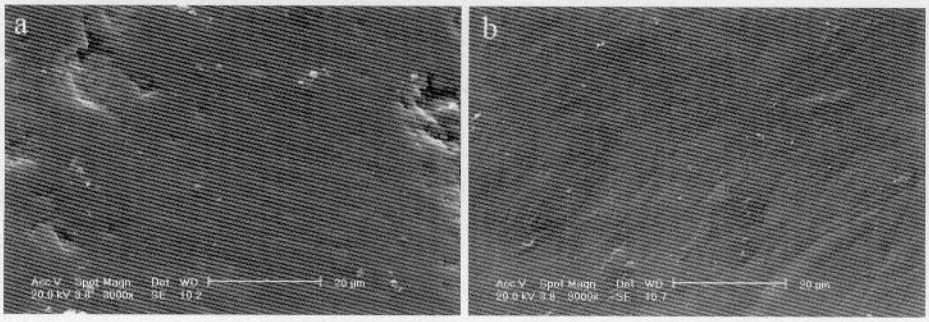

Fig 2. *Control (**a**) and experimental (**b**) enamel from group 2. In this case, some pores and superficial irregularities are evident on control enamel (a), while the surface of treated specimen (b) appears smooth.*

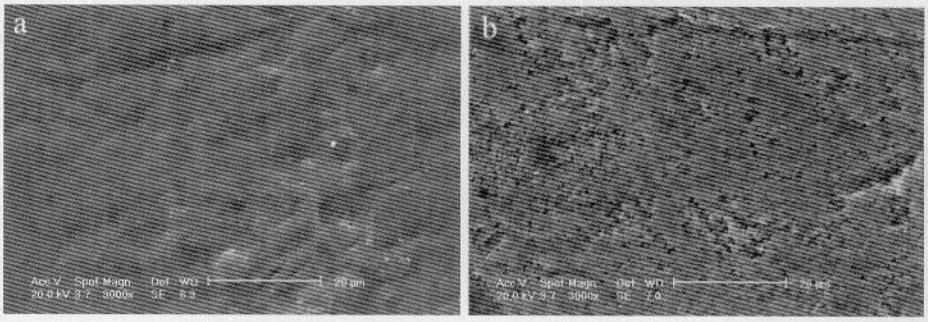

Fig 3. *Control (**a**) and experimental (**b**) enamel from group 3. Significant changes of enamel surface morphology can be appreciated. The surface of treated enamel (b) shows an important dissolution aspect.*

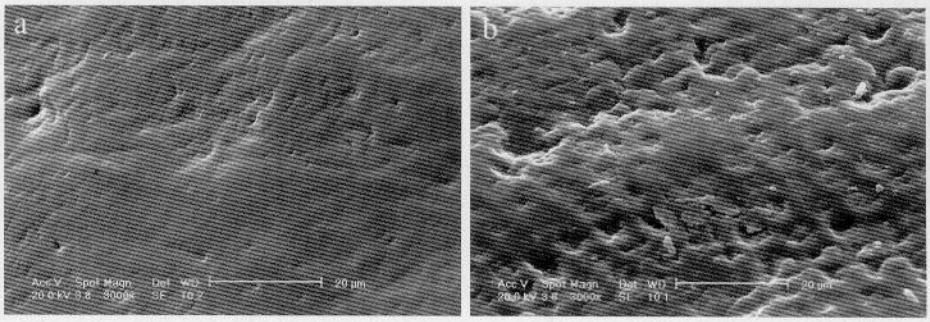

Fig 4. *Control (**a**) and experimental (**b**) enamel from group 4. Important alterations of the prismatic structure of the enamel with exposure of the enamel prisms can be observed on the treated surface (b).*

clinical report available on the potential development of caries from a broad series of whitening treatments. Nevertheless, only in recent years "tooth whitening mania" has arisen (13) and it may be assumed that we may face the consequences of highly repeated applications of a professional bleaching regimen on the same patient in the near future.

A limit of the present study is that a SEM qualitative assessment of the results was performed on extracted teeth. As an *in vitro* study, the methods used do not accurately reflect the *in vivo* situation (18). It was suggested that the mineral content of the saliva and the presence of fluoride might act as a remineralizing agent for enamel and dentine (19).

An attempt to simulate oral conditions was done by storing the specimens in artificial saliva between active periods of bleaching protocols and before each SEM evaluation.

The pH of bleaching agents is closely related to the alterations of the enamel surface. According to several authors, the more neutral the bleaching agent's pH the slighter the enamel alterations will be (5, 20). In the present study, a bleaching agent with pH 7 was used. Thus, it is possible to state that the pH may influence in the action of bleaching agents onto hard tissues, although there are other factors, such as total contact time, which can cause the alteration of these tissues.

In conclusion, from the results of the present study, the concept that "in-office" bleaching is a non-destructive cosmetic procedure should be reconsidered. It should be considered that enamel demineralization could be an undesirable effect of bleaching agents, related to the number of bleaching applications and to the time necessary to obtain teeth whitening.

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