ORIGINAL PAPER

The use of scanning electron microscopy in evaluating the effect of a bleaching agent on the enamel surface

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Abstract

In this paper we present the results of an experiment with a commercial gel containing carbamide peroxide (CP) 15% (Opalescence 15% PF), aimed to assess the effects of this bleaching agent on the enamel surface of extracted human teeth, by using scanning electron microscopy (SEM). Opalescence 15% PF was applied on two quadrants of twelve extracted permanent, decay-free, human teeth, for 3 hours or 8 hours; for four teeth the application was performed once, while for eight teeth the treatment was repeated daily for 14 days (in order to simulate the usual clinical protocol of at-home bleaching). One quadrant of each tooth was used for control and the remaining quadrant was etched for 40 seconds with a gel containing 37% phosphoric acid. No differences concerning the micromorphology of tooth surface were observed by SEM, on the samples treated with Opalescence 15% PF once, for 3 or 8 hours, when compared to the control. Various, mainly minor changes occurred in samples treated with Opalescence 15% PF for 3 hours or 8 hours daily, for 14 days. On the other hand, the acid-etched samples had an irregular surface, which suggests important alterations of the prismatic structure of the enamel. These are the first studies of this type performed in Romania.

***Keywords*:** tooth bleaching, enamel, carbamide peroxide, scanning electron microscopy.

Introduction

The appearance of natural teeth results from many optical phenomena – the basic color is mainly determined by the dentin, with the more translucent enamel playing an important role through scattering at wavelengths in the blue range [1].

Tooth color is not constant during lifetime; moreover, discoloration of the teeth can originate from a variety of intrinsic and extrinsic sources (reviewed in [2]). Intrinsic discolorations (irreversible pigmentation in varying colors and degrees of chromacity, which affect the structure of the dentin and/or of the enamel) are caused by pulpal trauma (which result in hemoragic products), by genetic and congenital disorders (e.g. phenylketonuria, congenital hyperbilirubinemia, amelogenesis and dentinogenesis imperfecta), or by medication (e.g. tetracycline, fluorosis).

Yellow-to-brown superficial, extrinsic, discolorations affect only the enamel surface; they occur because of consuming highly colored foods or beverages, smoking, or poor oral hygiene. Discoloration can also accompany aging because of the cumulative effects of surface strains and enamel thinning.

Noticeable discoloration of teeth should not be regarded only as a condition of cosmetic importance, since it can become a physical handicap with impacts on a person's self-image, self-confidence, attractiveness and employability [3]. Therefore, tooth-whitening (or bleaching) has become one of the most popular and successful aesthetic dental services offered to patients [2]. Intrinsic discolorations are sometimes difficult to treat; however, tooth-whitening procedures facilitate color modification in up to 97% of cases. This is why a dramatic increase in the public demand for bleaching lead to the development of various products and methods of applications. The bleaching agents can be applied externally (vital bleaching) or internally within the pulp chamber (non-vital bleaching).

The bleaching materials currently used have as the active ingredient either hydrogen peroxide (H_2O_2) or its precursor carbamide peroxide (CP), in various concentration. These bleach the chromogens within the enamel and dentine, thereby reducing the body color of the tooth [4, 5]. The result is the increasing in lightness and the decreasing in chroma [1]. The lightening effect is promoted by a higher concentration in H_2O_2 and/or an extended treatment time.

However, concerns have been expressed over the potential adverse effects of the use of H_2O_2 bleaching agents: cervical root resorption associated with non-vital bleaching, or increased tooth sensitivity and alteration in the surface texture and topography of enamel, hardness,

wear resistance and chemical composition of enamel and dentin associated with vital bleaching. In addition, reduction in bond strength of resin based materials and the possibility that H_2O_2 may have carcinogenic or tumor-promoting capabilities have been considered (reviewed in [6]).

There are conflicting results regarding the effects of bleaching materials containing CP on the enamel [7–11]. In order to try to solve this controversy we designed *in vitro* experiments exposing teeth to a commercial gel (Opalescence 15% PF) followed by the examination of the micromorphology of the enamel surface by scanning electron microscopy (SEM). These are the first studies of this type performed in Romania.

The aim of this paper was to assess, by using SEM, the effects of a bleaching agent based on CP (Opalescence 15% PF), on the enamel surface of human teeth.

Material and Methods

Twelve extracted permanent, decay-free, human teeth were used in these experiments. The anatomical root was covered in self-curing acrylic resin (Duracryl, Spofa Dental, Prague, Czech Republic), and the anatomic crown was sectioned bucco-lingual and mesio-distal, into four quadrants, with a low speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, USA), under water lubrication. The crown quadrants were separated from the roots through a basal section.

Two experimental protocols were designed. In the first protocol, applied to four teeth, one quadrant (labeled A) was used as a control. Two quadrants (labeled as B and C) were treated with bleaching gel based on CP (Opalescence 15% PF, Ultradent Products, South Jordan, USA) for 3 and 8 hours respectively. The last quadrant (D) was etched for 40 seconds with a gel containing 37% phosphoric acid.

In the second protocol, applied to eight teeth, the quadrants A were the controls; quadrants D were treated as in the first protocol, while the treatment of quadrants B and C with Opalescence 15% PF for 3 hours and 8 hours respectively was repeated daily, for 14 days. Between the active periods of bleaching gel application, the teeth quadrants were stored in artificial saliva (KCl 50 mmol/L, Ca^{2+} 1.5 mmol/L, PO_4^{3-} 0.9 mmol/L, tri-hydroxymethylamino-methane buffer 20 mmol/L, at pH 7.0) [12, 13].

After the treatment, the teeth quadrants were thoroughly cleaned and prepared for scanning electron microscopy (SEM) examination according to the usual protocols [14–16]. We used aluminum stubs as holders on which the specimens were fastened using carbon double-sided adhesive tabs (Electron Microscopy Sciences, Hatfield, USA), of 9 mm diameter, which were also used to establish a conductive pathway to the underlying metal stub. In order to ensure uniform electrical and thermal conductivity of the teeth surface we used colloidal silver (Polaron Equipment Ltd., Watford, UK) applied as a thin, continuous line around each tooth quadrant, at the level of the basal section, in contact with the carbon tab (Figure 1).

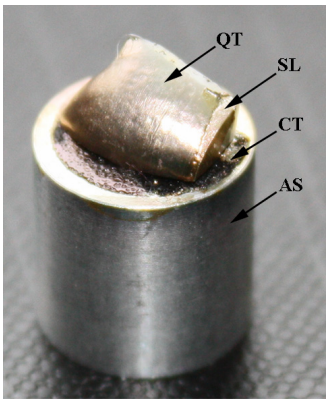

Figure 1 – ***A quadrant of a tooth (QT) placed on an aluminum stub (AS), using a carbon tab (CT) and a silver line (SL).***

The samples were then placed in a Polaron E–5100 plasma-magnetron sputter coater (Polaron Equipment Ltd., Watford, Hertfordshire, UK) and maintained over night at low vacuum (0.5 torr) for desiccation. The next day the samples were coated with gold in a high-purity argon gas at low pressure (0.05 torr), for 1 minute at 2 kV and 20 mA, in order to cover the samples with a thin layer of gold atoms of around 3–5 nm thickness.

The samples were examined with a JEOL JSM–25 Scanning Microscope (Jeol Ltd. Tokyo, Japan), at 30 KV acceleration voltage and magnifications of 200× and 2000×. The images – three for each quadrant of each tooth – were captured with a Deben Pixie–3000 image processor (Deben UK Ltd., Debenham, Suffolk, UK). The images, viewed on a monitor, were evaluated by four examiners (I–IV) and scored as follows: (1) smooth, normal enamel; (2) fissures on the enamel surface; (3) images of mildly increased porosity; (4) images of exposed enamel prisms and dissolution type I–III (see "Discussion").

Each examiner scored all the three images of the quadrants of each tooth; the mean score (the mean of the three images scored by each examiner) was included into Tables 1 and 2. In the first experiment, for the calculation of the statistical significance of changes in the enamel surface of samples B and C the final mean of scores for all four teeth was used. In the second experiment, for the calculation of the statistical significance of changes in the enamel surface of samples B and C the final mean of scores for all eight teeth was used.

The statistic analysis was performed using paired samples *t*-test (significance at $p<0.05$).

Results

Typical SEM aspects of the dental enamel surface of teeth used in these experiments are presented in Figures 2–7.

Figure 2 depicts unbleached enamel. The surface is not completely smooth, however the aprismatic surface layer is uniform. Pores and superficial irregularities, such as grooves can be observed, on some of the control samples. In such a case, samples were labeled with score 2. For the teeth used in the first experiment, the mean scores for the control quadrants varied between 1.33–2.00 (Table 1) and for the teeth used in the second experiment, the mean scores for the control quadrant varied between 1.00–2.00 (Table 2).

Table 1 – ***The scores of SEM images for samples of the four teeth used in the first protocol described in "Material and Methods"***[a]

Quadrant	Examiner	Tooth number and score				Final means of scores	Statistical significance (*t*-test)
		D1	*D2*	*D3*	*D4*		
A	I	2.00	1.66	2.00	2.00	1.92	
	II	1.33	1.33	1.33	2.00	1.50	
	III	1.33	1.33	1.66	2.00	1.58	
	IV	2.00	2.00	2.00	2.00	2.00	
B	I	2.00	2.33	2.66	2.66	2.41	
	II	2.00	1.00	2.00	2.00	1.75	0.166056
	III	1.33	1.66	1.33	1.66	1.50	
	IV	2.33	2.33	2.66	2.66	2.50	
C	I	2.00	2.33	2.33	2.66	2.33	
	II	1.66	1.66	2.00	1.66	1.75	0.153113
	III	1.66	2.00	1.33	1.66	1.66	
	IV	2.00	2.33	2.33	2.00	2.17	
D	I	4.00	4.00	4.00	4.00	4.00	
	II	4.00	4.00	4.00	3.66	3.92	0.000001
	III	4.00	4.00	4.00	4.00	4.00	
	IV	4.00	4.00	4.00	4.00	4.00	

[a]The significance of quadrants is: A – control; B – treated with Opalescence 15% PF for 3 hours; C – treated with Opalescence 15% PF for 8 hours; D – treated with H_3PO_4 for 40 seconds. For each quadrant, the score is the mean of three images scored by each examiner (I–IV). For the calculation of statistical significance of changes in the enamel surface of samples B and C the final mean of scores for all eight teeth was used. Other details are described in "Material and Methods".

Table 2 – ***The scores of SEM images for samples of the eight teeth used in the second protocol described in "Material and Methods"***[a]

Quadrant	Examiner	Tooth number and score								Final means of scores	Statistical significance (*t*-test)
		D1	*D2*	*D3*	*D4*	*D5*	*D6*	*D7*	*D8*		
A	I	2.00	2.00	2.00	2.00	2.00	2.00	2.00	1.66	1.96	
	II	1.66	2.00	2.00	2.00	2.00	2.00	2.00	2.00	1.96	
	III	1.66	2.00	2.00	2.00	1.66	2.00	2.00	2.00	1.92	
	IV	1.00	2.00	2.00	1.66	1.66	1.66	2.00	2.00	1.75	
B	I	2.33	2.00	2.33	2.33	3.00	2.33	2.33	2.66	2.14	
	II	2.33	2.00	2.00	2.33	2.66	2.00	2.33	2.33	2.25	0.0091665
	III	2.33	2.00	2.00	2.33	2.33	2.00	2.00	2.00	2.12	
	IV	2.00	2.00	2.00	2.00	2.00	1.66	2.00	2.66	2.00	
C	I	2.33	2.66	2.00	2.00	3.00	2.33	2.33	2.00	2.33	
	II	2.00	3.00	2.00	2.00	2.00	2.00	2.00	2.00	2.13	0.0055835
	III	2.00	3.00	2.00	2.00	2.00	2.00	2.00	2.00	2.13	
	IV	2.00	2.66	2.00	2.00	2.00	2.00	2.00	2.00	2.08	
D	I	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	
	II	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	0.00000001
	III	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	
	IV	4.00	4.00	4.00	4.00	3.66	4.00	4.00	4.00	3.96	

[a]The significance of quadrants is: A – control; B – treated with Opalescence 15% PF for 3 hours daily for 14 days; C – treated with Opalescence 15% PF for 8 hours daily for 14 days; D – treated with H_3PO_4 for 40 seconds. For each quadrant, the score is the mean of three images scored by each examiner (I–IV). For the calculation of statistical significance of changes in the enamel surface of samples B and C the final mean of scores for all eight teeth was used. Other details are described in "Material and Methods".

It appeared that a single exposure of teeth to Opalescence 15% PF, for 3 hours or 8 hours, does not change the structure of enamel. For quadrants B, the mean of scores varied between 1.00–2.66 and the difference between the studied group and the control group was not statistically significant (p=0.166056). For the quadrants C the mean of scores varied between 1.33–2.66 and the difference was also not statistically significant (p=0.153113).

In case of samples treated with Opalescence 15% PF, in a single session, 3 and 8 hours respectively (Figures 3 and 4), there were no considerable differences concerning the micromorphology of the enamel surface compared to the control samples, as displayed in Figure 2.

Table 2 presents the scores of SEM images for the eight teeth used in the second protocol.

In the second experiment, for the quadrants B the mean of scores varied between 1.66–2.66 and the difference from control was statistically significant (p=0.0091665). For the quadrants C the mean of scores varied between 2.00 and 3.00 and the difference was statistically significant (p=0.0055835).

Morphological changes were observed in some of

the samples treated with Opalescence 15% PF, for 3 hours or 8 hours daily, for 14 days, consisting in areas of depressions which seem sometimes deeper, generating a more variable aspect of the enamel surface (Figures 5 and 6). This aspect suggests an increase in the enamel porosity, as compared to the control samples. However, areas of depression were observed sometimes in the control samples.

On the other hand, the acid-etched samples had in all the cases an irregular surface, which suggests important alterations of the prismatic structure of the enamel (Figure 7).

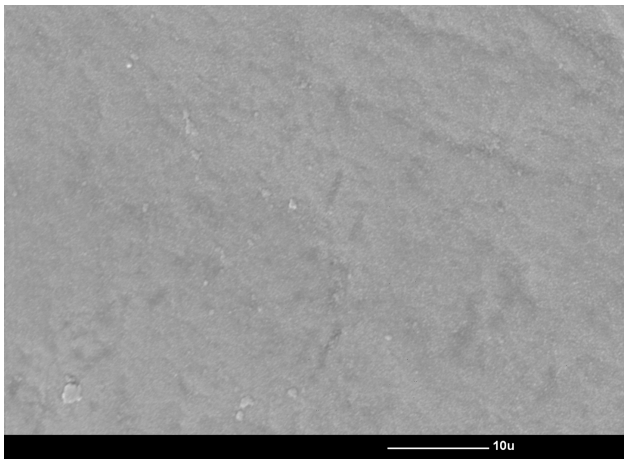

Figure 2 – ***The SEM micrograph of enamel surface of a control tooth sample (quadrant A, as described in "Material and Methods").***

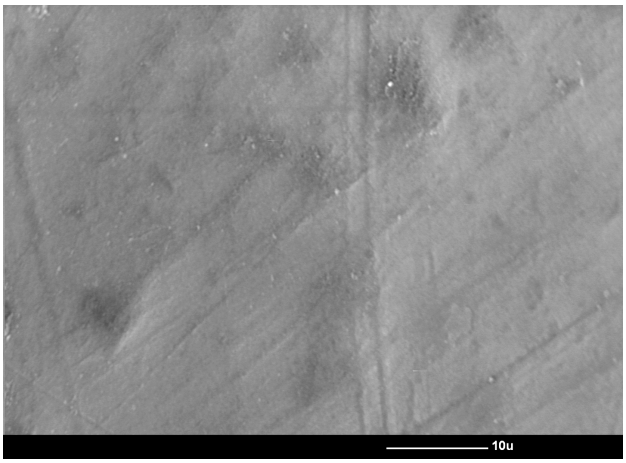

Figure 3 – ***The SEM micrograph of enamel surface of a tooth sample treated with Opalescence 15% PF for 3 hours.***

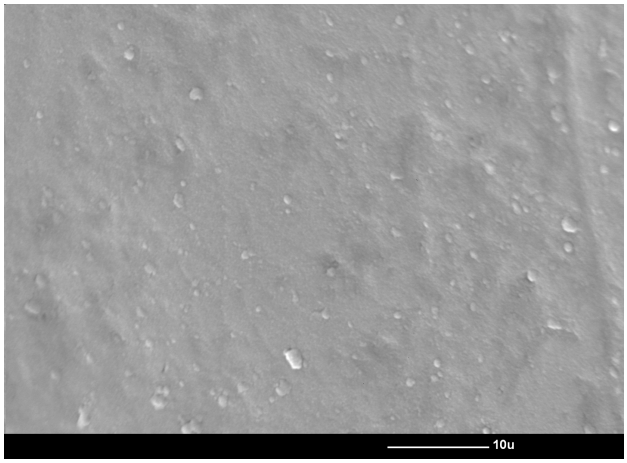

Figure 4 – ***The SEM micrograph of enamel surface of a tooth sample treated with Opalescence 15% PF for 8 hours.***

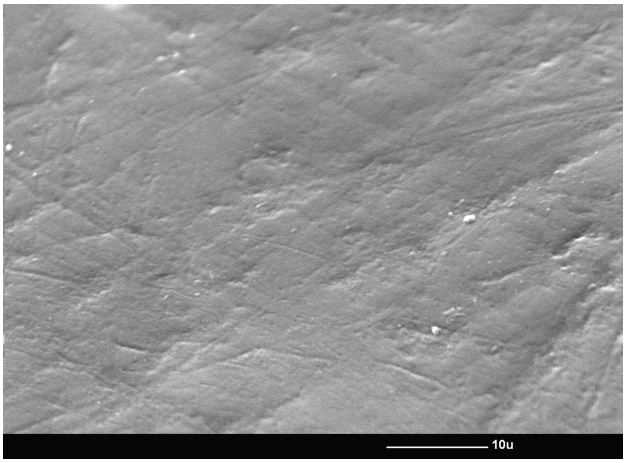

Figure 5 – ***The SEM micrograph of enamel surface of a tooth sample treated with Opalescence 15% PF 3 hours daily for 14 days.***

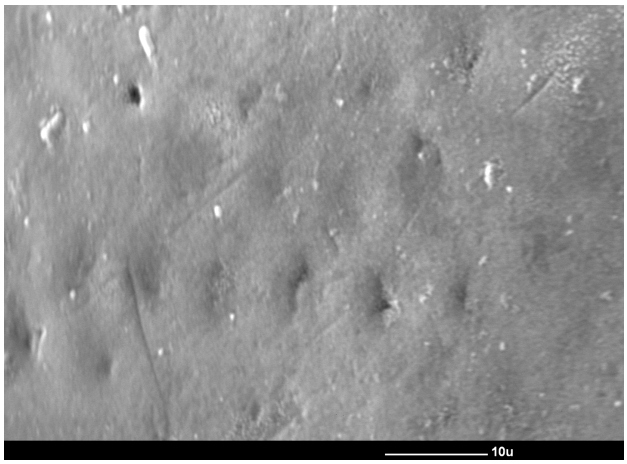

Figure 6 – ***The SEM micrograph of enamel surface of a tooth sample treated with Opalescence 15% PF 8 hours daily for 14 days.***

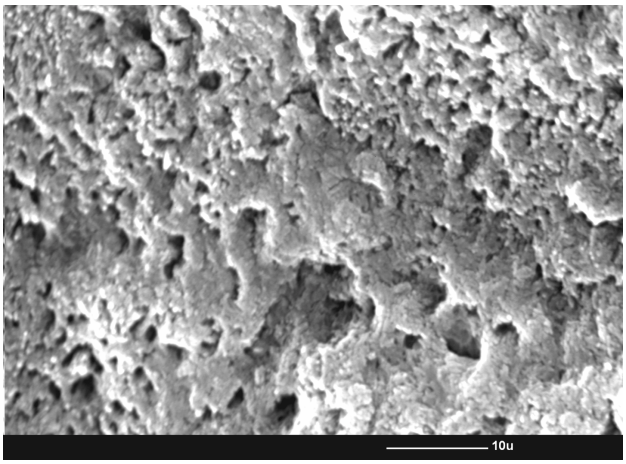

Figure 7 – ***The SEM micrograph of enamel surface of a sample treated for 40 sec with a gel containing 37% phosphoric acid (quadrant D, as described in "Material and Methods").***

The mean scores attributed to the images corresponding to the quadrants D varied in both experiments between 3.66–4.00, and the statistical significance of the difference of means compared to the control group was very high (p=0.000001 and p=0.00000001, respectively).

In our study, the pattern of demineralization followed either a version in which the central part of the prism was involved, or an irregular meshwork.

Discussion

The whitening effect of the material used in this study (Opalescence 15% PF) is achieved by decomposing CP into urea and H_2O_2, and the morphological changes of teeth have been attributed to these degradation by-products [3, 4, 6]. Urea denatures the proteins in the organic content of dental structures, with the potential to penetrate through enamel and affect the prismatic and interprismatic structures contributing to the permeability increase and microstructural changes (Arends *et al.*, 1984, Goldberg *et al.*, 1983, cited by [6]). H_2O_2 is converted to perhydroxyl anion (HO_2^-) and free radicals, which destroy or oxidize the double bonds in the conjugated chain of chromophore [5]. Both enamel and dentin are permeable by H_2O_2 and CP; it was suggested that the peroxides could even reach the pulp chamber (Suleiman *et al.*, 2005, cited by [4]). Thus bleaching is not achieved solely by a surface effect [3], however the adverse effects of bleaching materials on the enamel surface are essential. Opalescence also contains sodium fluoride (F) and potassium nitrate (PN). F reduces sensitivity by blocking dentin tubules and slowing fluid movement, while PN has an analgesic effect on the transmission of nerve impulses (reviewed in [2]).

One of the best methods to study the enamel surface is SEM. There are conflicting reports regarding the effects of bleaching materials containing CP on the enamel. Some authors reported morphological changes of the enamel surface after exposure of human teeth to CP gels, such as focal areas of shallow erosion, loss of the prismatic layer, pitting, and exposure of the enamel prisms; moreover, it was suggested that after a prolonged exposure to increased concentration of CP (35%) or H_2O_2 (35%) such changes could be the cause of abrasion or cusp fractures, mainly in restored, weakened teeth [8, 10, 11]. It was stated that neither the patient, nor the clinician is in the position to control the degree of penetration by the bleaching agent of the dental structures subjected to its action. Other authors found minimal effects of CP on the surface micromorphology of enamel [9].

In our study, we examined by SEM the enamel surface after a single application of a 15% CP bleaching agent vs. a regimen of repeated applications for 14 days (which simulates the usual at-home bleaching). Some differences were noticed between the two regimens (single exposure and 14 exposures): minor changes of the enamel surface occurred in samples treated with Opalescence 15% PF, for 3 hours or 8 hours daily, for 14 days. Our results are similar to other studies, which found minor changes on the surface micromorphology of enamel induced by the 14 days regimen.

On the other hand, these irregularities are difficult to be considered as secondary effects of the treatment. When the enamel surfaces were examined in the control groups, sometimes pores, shallow depressions and superficial irregularities are observed, but this situation was reported by other authors, as well [10]. On the surface of normal, sound teeth, circumferentially horizontal lines, known as perikimata, may be found across the face of the crown; on the other hand, lamellae or cracks are not unusual. Electron microscopy observations showed that the surface of the enamel varies with age [17].

Furthermore, when bleached teeth were compared with teeth treated with placebo, in studies performed *in vivo* [7] the same minimal altered morphology was observed. It was suggested that other factors – the patient's diet or oral hygiene habits might be responsible by these irregular surface of the enamel [9, 18, 19].

In our study permanent, decay-free, adult erupted teeth were used, extracted for periodontal reasons; this may explain the occasionally fissures on the enamel surface, aspects which were not influenced by the exposure to bleaching agents, since they were observed on the control samples as well.

The scale we used for coding the images of the enamel surface was a modified version of the model presented by Basting RT *et al.* [7], which is a yes–no score system, based on the recorded changes. Our score system is a more complex one, which allows a quantification of the changes and tries to identify the enamel samples, which presents defects, which are not induced by the treatment.

Another limit of our study is that we used extracted teeth, even if we tried to simulate the oral condition, by storing the samples in artificial saliva during active period of treatments. 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 [18, 20, 21]. The studies in the literature that do show an effect on the enamel surface, in general, have some limitations in the *in vitro* methods used which do not accurately reflect the *in vivo* situation [22].

The 37% phosphoric acid is used in dentistry for etching the enamel and dentine in order to enhance the adhesion of the composite resins. It generates a porous surface, due to the selective dissolution of apatite crystals from the enamel prisms. According to the orientation of the dissolved crystals, several pattern of etching are described: type I, in which the crystals belonging to the center of the enamel prisms are involved, type II, generated by the dissolution of the crystals from the periphery of the prism, while type III is associated with an irregular pattern of demineralization [17].

In our study, a quadrant of each tooth was treated with 37% etching, in order to observe the aspect of the etched enamel, as compared to the bleached surfaces. Important differences were noted between the two types of treatment.

Conclusions

No differences concerning the micromorphology of tooth surface were observed by SEM, on the samples treated with Opalescence 15% PF once, when compared to the control.

Minor changes, statistically significant, occurred in samples treated with Opalescence 15% PF for 3 hours and for 8 hours daily for 14 days.

On the other hand, the acid-etched samples had an irregular surface, which suggests important alterations of the prismatic structure of the enamel.

Acknowledgements

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