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Effect of Two In-office Whitening Agents on the Enamel Surface *In Vivo*: A Morphological and Non-contact Profilometric Study

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Clinical Relevance

This *in vivo* study supports the hypothesis that the use of in-office bleaching agents is a safe and reliable procedure, inducing no structural damage to the enamel surface, even after prolonged and repeated applications.

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SUMMARY

This study evaluated the morphological effects produced *in vivo* by two in-office bleaching agents on enamel surface roughness using a non-contact profilometric analysis of epoxy replicas. The null hypothesis tested was that there would be no difference in the micromorphology of the enamel surface during or after bleaching with two different bleaching agents. Eighteen subjects were selected and randomly assigned to two treatment groups (n=9). The tooth whitening materials tested were 38% hydrogen peroxide (HP) (Opalescence Xtra Boost) and 35% car-

bamide peroxide (CP) (Rembrandt Quik Start). The bleaching agents were applied in accordance with manufacturer protocols. The treatments were repeated four times at one-week intervals. High precision impressions of the upper right incisor were taken at baseline as the control (CTRL) and after each bleaching treatment (T_0: first application, T_1: second application at one week, T_2: third application at two weeks and T_3: fourth application at three weeks). Epoxy resin replicas were poured from impressions, and the surface roughness was analyzed by means of a non-contact profilometer (Talysurf CLI 1000). Epoxy replicas were then observed using SEM. All data were statistically analyzed using ANOVA and differences were determined with a *t*-test. No significant differences in surface roughness were found on enamel replicas using either 38% hydrogen peroxide or 35% carbamide peroxide *in vivo*. This *in vivo* study supports the null hypothesis that two in-office bleaching agents, with either a high concentration of hydrogen or carbamide peroxide, do not alter enamel surface roughness, even after multiple applications.

INTRODUCTION

The demand by patients for esthetic treatments has definitely increased, with the whitening of discolored teeth becoming a popular procedure. Vital bleaching with carbamide and hydrogen peroxide can be performed on external enamel using an at-home technique (nightguard vital whitening) or with highly concentrated bleaching agents that are available for in-office procedures (in-office power whitening).[1-3] Previous studies investigated peroxide concentration, time and mode of application to achieve the highest whitening efficacy in relation to different clinical situations.[1-3]

The current opinion is that tooth whitening is an effective clinical treatment. Nevertheless, the side effects of bleaching agents on dental tissues have not been completely resolved. The issue of morphological enamel surface alterations following bleaching is controversial, despite the fact that a large number of studies have investigated the possible formation and continuation of those alterations (Table 1).[4-42] However, an overwhelming majority of morphological studies have been performed *in vitro*, frequently leading to different results in relation to different testing conditions, morphological aspects and the mechanical properties challenged. The major differences between these studies are the type of study setup (*in vitro* vs *in vivo*), sample size, type of tissue (human or bovine enamel), preparation of the tissue (polished or sound enamel), type of analysis carried out (mechanical test or morphological analysis), bleaching agent used (hydrogen peroxide or carbamide peroxide), bleaching agent concentration and formulation (gel or solution), length of bleaching agent exposure and other evaluation criteria. Also, methods of analysis are different among the studies, with protocols based on scanning electron microscope (SEM) analysis,[20-21,27,30-31,34,36,38] microhardness tests,[9,11,14,36,38,40] profilometric techniques,[4,15,18,34,36,38-39] plasma-atomic emission spectrometric analysis associated with chromatography,[37] infrared absorption spectroscopy correlated with x-ray analysis,[41] atomic force microscopy[10,28] and nanoindentation techniques.[5]

Conversely, only a few studies have attempted to assess whitening effects *in vivo*,[22,26,29] usually based on the analysis of an enamel replica using SEM. These studies on enamel surface characteristics[22,26,29] are mainly based on the morphologically subjective assessments of the enamel surface, rather than on precise measurements of the enamel surface profile. Since it has been demonstrated that *in vitro* alterations are more evident than *in vivo* alterations,[13] several studies support the hypothesis that factors, such as the remineralizing potential and the buffering ability of saliva, may counteract the adverse effects of whitening on the enamel surface.[17,43-44] For this reason, analysis of the enamel surface profile after bleaching *in vivo* is worthy of investigation. As currently researched, no previous studies on enamel roughness using a non-contact profilometer after bleaching *in vivo* have been published.

This study evaluated the effect of two high-concentration in-office bleaching agents applied *in vivo* on the enamel surface. The null hypothesis tested was that the whitening procedures would not alter the surface roughness of enamel.

METHODS AND MATERIALS

Eighteen subjects (5 male, 13 female) volunteered for the study (age 21-35 years, mean 25 years). Signed informed consent was received from the patients under a protocol that was approved by the University of Trieste, Trieste, Italy. All subjects had anterior tooth shades A3 or darker, as determined using the Vita Classical shade guide (Vita Zahnfabrik, Bad Säckingen, Germany). Inclusion criteria were the presence of all maxillary incisors and canines; the absence of caries, restorations and periodontal disease; no previous tooth whitening treatment; the absence of smoking habits and compliance to procedures to avoid staining from food and beverages (tea, coffee, licorice, red wine, etc) during the treatment period.

The subjects underwent a professional prophylaxis one week before starting the study and were given oral hygiene instructions: tooth brushing twice a day with an Elmex InterX Sensitive toothbrush (Gaba International AG, Münchenstein, Switzerland) using a low abrasive toothpaste (Elmex Sensitive Plus, Gaba International AG, RDA value = 30) and dental flossing once a day.

Table 1: *Literature Review of the Studies on the Whitening Effects on Enamel*

Authors	Substrate	Study Design	Whitening Agents	Technique of Analysis	Alterations
Akal & others, 2001	Human incisors	*In vitro*	10% carbamide peroxide	Microhardness, SEM	yes
Attin & others, 2004	Bovine incisors	*In vitro*	10% to 15% to 35% carbamide peroxide; 6% hydrogen peroxide	Microhardness, Fracture toughness	yes
Attin & others, 2005	Bovine crowns	*In vitro*	10% to 15% carbamide peroxide	Microhardness	yes
Basting & others, 2001	Human enamel	*In vitro*	10% carbamide peroxide	Microhardness	yes
Basting & others, 2003	Human enamel	*In vitro*	10% to 22% carbamide peroxide	Microhardness	yes
Basting & others, 2005	Human enamel	*In vitro*	10% carbamide peroxide	Microhardness	yes
Bitter, 1998	Human enamel	*In vitro*	10% carbamide peroxide	SEM	yes
Cavalli & others, 2004	Human enamel	*In vitro*	35% to 37% carbamide peroxide	Profilometry, Spectrophotometric analysis, SEM	yes
Cimilli & Pameijer, 2001	Human enamel	*In vitro*	10% to 15% to 16% carbamide peroxide	Microhardness, Infrared spectrophotometry, FTIR, X-ray diffraction	yes
Cobankara & others, 2004	Human incisors	*In vitro*	10% to 15% carbamide peroxide	Profilometry, SEM	no
Efeoglu & others, 2005	Human molars	*In vitro*	10% carbamide peroxide	Microcomputerized tomography	yes
Ernst & others, 1996	Human enamel	*In vitro*	10% carbamide peroxide, 30% hydrogen peroxide, 30% hydrogen peroxide + sodium perborate	SEM	slight
Gultz & others, 1999	Human incisors	*In vitro*	35% carbamide peroxide, 38% hydrogen peroxide	SEM	no
Hairul Nizam & others, 2005	Human premolars	*In vitro*	30% hydrogen peroxide	Nanoindentation technique (Microhardness, Young's modulous)	yes
Hegedus & others, 1999	Human incisors	*In vitro*	10% carbamide peroxide, 30% hydrogen peroxide	Atomic Force Microscopy	yes
Hosoya & others, 2003	Human molars	*In vitro*	35% hydrogen peroxide	Profilometry, S mutans adhesion	yes
Justino & others, 2004	Human premolars	*In vitro*/*In vivo*	10% carbamide peroxide	Microhardness, Calcium dosage, SEM	yes *in vitro* no *in vivo*
Kwon & others, 2002	Bovine incisors	*In vitro*	30% hydrogen peroxide	SEM, UV-VIS-NIR spectrophotometry	yes
Lee & others, 1995	Human enamel	*In vitro*	35% hydrogen peroxide, 50% hydrogen peroxide	Microhardness, SEM	yes (50% hydrogen peroxide)
Lee & others, 2006	Bovine incisors	*In vitro*	30% hydrogen peroxide	Plasma-atomic emission spectrometer, Ion chromatography	yes
Leonard & others, 2001	Human incisors	*In vitro*	10% carbamide peroxide	SEM (replica technique)	slight
Lewinstein & others, 2004	Human molars	*In vitro*	35% hydrogen peroxide, 35% carbamide peroxide	Microhardness	yes
McGuckin & others, 1992	Human enamel	*In vitro*	10% carbamide peroxide, 30% hydrogen peroxide	SEM	yes
Moraes & others, 2006	Human molars	*In vitro*	10% and 35% carbamide peroxide	Profilometry	yes (35% carbamide peroxide)
Murchison & others, 1992	Human premolars	*In vitro*	10% carbamide peroxide	Microhardness, Bond strength	no
Nucci & others, 2004	Human enamel	*In vitro*	10% carbamide peroxide, 6% hydrogen peroxide	SEM	no
Oltu & Gürgan, 2000	Human molars	*In vitro*	10% to 16% to 35% carbamide peroxide	Infrared absorbtion spectroscopy, X-ray diffraction analysis	yes (35% carbamide peroxide)
Park & others, 2004	Bovine enamel	*In vitro*	30% hydrogen peroxide	FT-Raman, Microhardness	slight
Pinto & others, 2004	Human molars	*In vitro*	3% carbamide peroxide, 10% carbamide peroxide, 7.5% hydrogen peroxide; 35% carbamide peroxide; 38% hydrogen peroxide	Microhardness, Profilometry, SEM	yes
Rodrigues & others, 2005	Human enamel	*In vitro*	10% carbamide peroxide, 37% carbamide peroxide	Microhardness	yes
Rotstein & others, 1996	Human premolars	*In vitro*	10% carbamide peroxide, 30% hydrogen peroxide, sodium perborate	Histochemical analysis	yes
Shannon & others, 1993	Human enamel	*In vitro*/*In vivo*	10% carbamide peroxide	Microhardness, SEM	yes
Spalding & others, 2003	Human teeth	*In vitro*	10% carbamide peroxide, 35% hydrogen peroxide	SEM	no
Sulieman & others, 2004	Human molars	*In vitro*	35% hydrogen peroxide	Microhardness, SEM	no
Turkun & others, 2002	Human incisors	*In vitro*	10% carbamide peroxide	SEM (replica technique)	yes
Unlu & others, 2004	Human incisors	*In vitro*	10 % to 15% carbamide peroxide	Microhardness	no
Worschech & others, 2003	Human molar	*In vitro*	35% carbamide peroxide	Profilometry	no
Yeh & others, 2005	Human premolars	*In vitro*	10% carbamide peroxide	SEM	yes
Zalkind & others, 1996	Human premolars	*In vitro*	10% carbamide peroxide, 30% hydrogen peroxide, sodium perborate	SEM	yes

The subjects were randomly divided into two groups (n=9). The tooth whitening materials tested were a 38% hydrogen peroxide bleaching agent (HP) (Opalescence Xtra Boost, Ultradent Products, South Jordan, UT, USA) and a 35% carbamide peroxide bleaching product (CP) (Rembrandt Quik Start, Den-Mat Corporation, Santa Maria, CA, USA). Bleaching treatments were repeated four times at one-week intervals. Each application was performed under rubber dam isolation. The teeth were cleaned with a brush mounted on a low-speed contrangle handpiece under water irrigation in order to remove residual biofilms from the surface and to allow for intimate contact between the enamel and bleaching agent. The bleaching agents were applied in accordance with manufacturer protocols. HP was provided with two syringes: one syringe contained the activator, while the other contained hydrogen peroxide. Before use, the activator was mixed with the bleaching agent. The activated HP whitening gel was applied to the teeth for 10 minutes, while the CP gel, which was ready for use, was applied for 30 minutes. For both materials, one application of the bleaching agent was performed at each appointment. At the end of each treatment, the bleaching agent was removed and the treated teeth were thoroughly rinsed with air-water spray for 30 seconds.

High precision impressions were taken immediately after bleaching, using a polyvinyl siloxane based material (Elite H-D+ Putty and Light Body, Zhermack, Rovigo, Italy) and the double-impression technique. An initial putty impression was recorded and allowed to fully set. Then, a light body material was carefully applied both into the first impression (the first impression was used as a customized tray) and on the teeth of interest in order to obtain a very precise final impression. Impressions of the upper right incisor were taken at baseline (CTRL) and after each bleaching treatment (T_0: first application; T_1: second application, one week; T_2: third application, two weeks; T_3: fourth application, three weeks). Replicas were prepared by pouring the impressions with an epoxy resin mixed under vacuum (Eposs EL 20, Prochima, Pesaro, Italy).

Two non-carious third molars were extracted from two different patients (mean age 23 years) for orthodontic reasons. Their vestibular surfaces were etched with 37% orthophosphoric acid for 30 seconds immediately after extraction, and epoxy replicas of the etched surfaces were obtained using the same technique used *in vivo*. These replicas served as a positive control.

All replicas were analyzed using a non-contact profilometer (Talysurf CLI 1000, Taylor Hobson Ltd, Leicester, England) equipped with a chromatic length aberration gauge, providing highly accurate non-contact 3D measurements. In a non-contact profilometer, a white beam is focused on a surface through a lens with chromatic length aberration. Due to this aberration, the focus point is at a different z-position of different wavelengths. The reflected light is sent to a spectrometer through a pinhole. The spectrometer provides an intensity curve, based on the wavelength. The focused wavelength is the one corresponding to the maximum intensity. This technique allows one to obtain a vertical resolution of about 50 nm; the non-contact measurements assure sample integrity and work on transparent materials.

Five readings were recorded for each specimen. The roughness parameters evaluated were roughness aver-

Table 2: *Mean and standard deviation of the roughness parameters of two bleaching agents before (CTRL) and after bleaching (T_0: first application; T_1: second application, one week; T_2: third application, two weeks; T_3: fourth application, three weeks) and of the positive acid-etched control specimens.*

	Ra	Sp	Sv	Ssk
HP CTRL	1.72±0.56	7.71±1.60	4.97±1.00	0.48±0.19
HP T_0	1.70±0.40	6.93±1.18	4.16±0.46	0.46±0.16
HP T_1	1.65±0.48	6.82±1.40	5.25±0.98	0.41±0.22
HP T_2	1.94±0.64	8.60±2.02	4.70±0.79	0.38±0.15
HP T_3	1.37±0.51	6.87±1.00	4.76±0.20	0.37±0.14
CP CTRL	1.42±0.23	7.70±1.88	4.38±0.58	0.52±0.44
CP T_0	1.81±0.20	7.90±1.58	5.24±1.26	0.44±0.17
CP T_1	1.42±0.14	7.10±1.49	4.99±0.99	0.39±0.29
CP T_2	1.40±0.13	7.23±1.54	4.09±0.70	0.37±0.26
CP T_3	1.36±0.16	7.56±1.38	4.62±1.12	0.41±0.29
Pre-etching specimen	1.59±0.63	7.29±1.54	4.77±1.70	0.54±0.26
Post-etching specimen	3.36±1.16	10.56±2.38	6.62±1.12	0.84±0.29

No statistical differences were found between the controls and measurements at T_0, T_1, T_2, and T_3 ($p>.05$). Only the post-etching specimens revealed statistical differences with all previous tested specimens ($p<.05$).

Ra=roughness average; Sp=maximum profile peak height; Sv=maximum profile valley depth; Ssk=surface skewness.

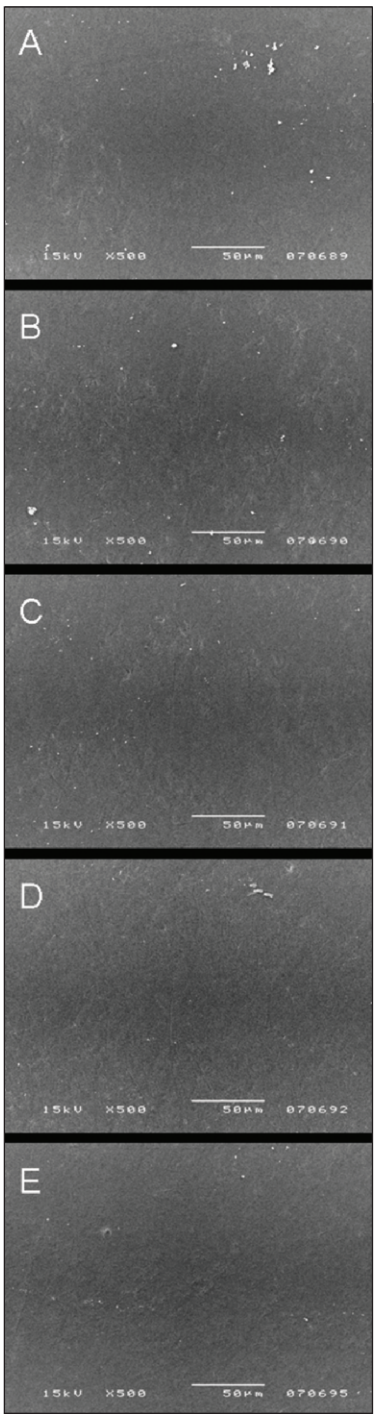

Figure 1: *SEM micrograph showing the enamel surface morphology treated with Opalescence Xtra Boost. A: enamel before bleaching application; B: T_0, first bleaching application; C: T_1, second bleaching application (one week); D: T_2, third bleaching application (two weeks); E: T_3, fourth bleaching application (three weeks); original magnification 500x. No significant morphological differences were found in relation to the bleaching treatment and timing of the treatment.*

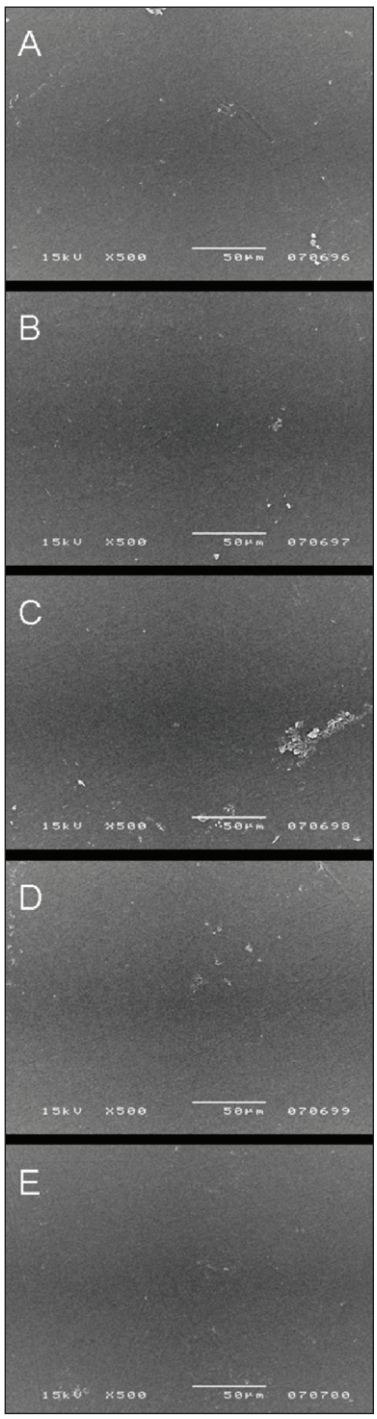

Figure 2: *SEM micrograph of human enamel treated with Rembrandt Quik Start. A: enamel before bleaching application; B: T_0, first bleaching application; C: T_1, second bleaching application (one week); D: T_2, third bleaching application (two weeks); E: T_3, fourth bleaching application (three weeks); original magnification 500x. No differences were found before or after application of the bleaching agent or in relation to the timing of the treatment.*

age (Ra); maximum profile peak height (Sp); maximum profile valley depth (Sv) and surface skewness (Ssk). Ra is the arithmetic mean deviation of the surface; Sq is the root-mean-square deviation of the surface; Sp defines the maximum height of summits: in particular, it defines the height between the highest peak and the mean plane; correspondingly, Sv is the parameter that identifies the depth between the mean plane and the deepest valley. Ssk describes the skewness of the height distribution. In particular, a negative Ssk indicates that the surface is composed with principally one plateau and deep and fine valleys. A positive Ssk indicates a surface with many peaks on a plane. An Ssk close to zero indicates a surface equally distributed.

Epoxy replicas were then sputter-coated with gold and observed using a scanning electron microscope (JEOL JSM–5200, Tokyo, Japan). Representative images of each specimen were taken by two independent observers with a blind study design. Both profilometric readings and SEM photographs were taken in the central third of the replicas.

Differences within each group were statistically analyzed with ANOVA for repeated measures. Differences between the groups were analyzed with a *t*-test. Statistical significance was preset at $p<0.05$.

RESULTS

After bleaching, both treatment groups demonstrated significant improvement in tooth color of at least two shades on the VITA shade guide.

Table 2 shows the mean and standard deviations of the roughness parameters of the two treatment groups before treatment (CTRL; unbleached enamel) and after each bleaching appointment at one week intervals (T_0, T_1, T_2 and T_3). No significant differences were found between the two groups or the positive control in the baseline values (Table 2).

Results demonstrated that the surface roughness of enamel did not increase after whitening with both bleaching agents at T_0, T_1, T_2 and T_3, because no significant differences in roughness parameters were revealed when compared to the controls. SEM analysis confirmed that the treated enamel surfaces were similar to enamel before bleaching in both groups (Figures 1A-E and 2A-2E). The small irregularities on the surface can be attributed to normal enamel topography.

Alternatively, the etched enamel surface showed a significant increase in roughness values, and the characteristic etching pattern

was demonstrated under SEM analysis (Figures 3A and 3B).

DISCUSSION

This is the first *in vivo* study performed using a non-contact profilometer to evaluate enamel roughness after tooth bleaching. The results of the current study indicate that in-office bleaching performed with Opalescence Xtra Boost (38% hydrogen peroxide, [HP]) or Rembrandt Quik Start (35% carbamide peroxide, [CP]), with the application repeated up to four treatments in four weeks, did not produce significant alterations on enamel surface roughness. The null hypothesis tested in this study was accepted.

This study was based on an epoxy resin replica technique[45] that provided the advantage of evaluating the effects of bleaching agents on enamel surface morphology under normal intraoral conditions,[22] while permitting morphological evaluation of the same area of the tooth before and after bleaching.[22] The method sensitivity was assessed by the positive control: the profilometer was able to detect the roughness increase of the etched enamel, confirming the typical etching pattern exhibited with SEM analysis. Surface roughness depends on the cut-off length used for the analysis. In this study, the authors performed surface scans of 0.5 x 0.5 mm in dimension, a suitable dimension for the phenomenon under investigation. In this study, it was decided that the cut-off length would be about half the analysis length. For this reason, the authors obtained etched enamel roughness values that were lower if compared to other recent studies[46] but higher if compared with different settings.[47]

Two high concentration in-office whitening agents were tested with the hypothesis that higher concentrations would lead to more morphological alterations than lower concentration products. Thirty eight percent hydrogen peroxide is one of the highest concentrated bleaching products commercially available, as it has a strong oxidizing effect, making it potentially harmful to enamel, as this tissue is susceptible to hydrogen peroxide. Thirty five percent carbamide peroxide is the highest concentration of carbamide peroxide available on the market, corresponding to 11.4% hydrogen peroxide and containing urea. It has been reported that urea, which breaks down to carbon dioxide and ammonia, may affect the interprismatic regions of enamel.[48-49] Urea could denature the protein structure and might cause structural and morphological alterations of enamel through the degradation of organic molecules, such as amelogenin.[28] On the other hand, since urea is alkaline, it raises the pH of the bleaching products, thus reducing the demineralization potential.[15]

In previous studies (Table 1), most *in vitro* studies using high concentrations of hydrogen peroxide solution have been reported to produce morphological alteration of the enamel surface,[15,21,30-32,34,38] modifications to enamel crystal distribution,[41] increased porosities of the superficial enamel structure[34] and higher adhesion of *S mutans* to the enamel surface.[18] Furthermore, surface chemical analysis has reported both modification in the calcium/phosphate ratio[42] and calcium loss,[37] thus supporting the hypothesis that bleaching agents are chemically active components able to induce important structural alterations of human enamel. The surface alteration of enamel was indirectly confirmed by studies reporting reduced enamel physical properties, among which microhardness was thoroughly investigated.[5,9-11,14,38,40]

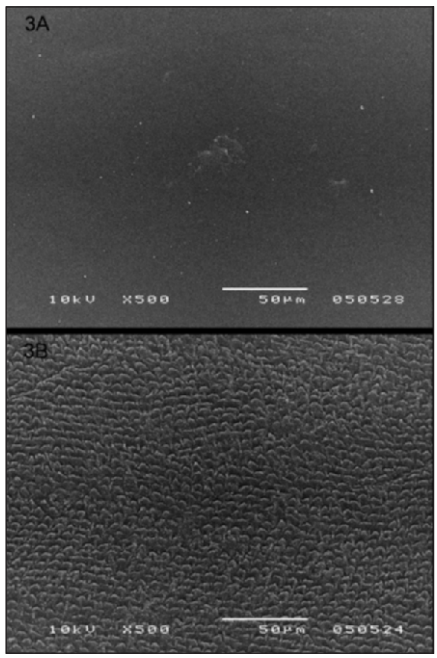

Figure 3: *SEM image of positive controls of the control group: A—enamel before etching; B—enamel after etching with 37% phosphoric acid gel for 30 seconds (magnification 500x).*

Since all of the evaluated profilometric parameters were not modified after whitening, this *in vivo* study demonstrated that a four-appointment regimen of in-office bleaching using both 38% hydrogen peroxide and 35% carbamide peroxide had no effect on the surface roughness of enamel. This would indicate that the appropriate use of high concentration bleaching products has no detrimental effect on enamel surface micromorphology. These results are in accordance with previous *in vitro* studies.[10,27,36,39]

Since both products tested in this experiment are designed for chairside procedures, they were used under rubber dam isolation. Impressions were recorded immediately after bleaching and before removing the rubber dam, thus, no contact with saliva was allowed. Therefore, the remineralizing effect of saliva on

bleached enamel surfaces[17,22,40-41,42-44] cannot be the reason for the absence of alterations observed in this study. One study has reported that surface alterations can be ascribed to the acidic characteristics of the bleaching agents.[36] The absence of morphological and profilometric changes on the enamel surface after *in vivo* bleaching treatments may be due to the relatively neutral pH of the tested products (HP, pH=7.0-7.5[50] and CP, pH= 6.5[51]), well above the critical value for enamel demineralization. This may also explain why no increase in enamel roughness was observed compared with enamel etched with 37% phosphoric acid.

The controversial outcomes of tooth whitening studies can be due to diversity in the experimental setups, making a comparison between results very difficult, if not impossible. Spalding and others[20] demonstrated that alterations in the tooth surface from the normal variation of enamel morphology may be higher than those alterations ascribed to the effect of peroxides on teeth. Additionally, the clinical significance of enamel alterations after bleaching, which have been reported in some studies, has not been clarified.[22,32,40]

Based on the results of the current study, the null hypothesis was accepted. Both HP and CP in-office bleaching products did not affect enamel surface roughness. However, previous studies suggest that subsurface alterations may occur in human teeth submitted to bleaching[9,21,34] due to the penetration of peroxide compounds into the enamel. Therefore, these observations and their clinical significance must be further investigated in in-office bleaching agents and at-home bleaching products, which, even if used at lower concentration, are kept in contact with enamel for a longer period of time.

CONCLUSIONS

No morphological changes were found on enamel surfaces using non-contact profilometric or SEM analysis. This *in vivo* study shows that in-office bleaching with a high concentration of hydrogen or carbamide peroxide is a safe, reliable procedure, inducing no structural changes to the enamel surface after four applications.

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