Original Article

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Permeability, roughness and topography of enamel after bleaching: tracking channels of penetration with silver nitrate

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Abstract

Aim: This study evaluated the surface roughness, topography and permeability of bovine enamel by profilometry and scanning electron microscopy (SEM) with and without silver nitrate solution, after exposure to different bleaching agents. **Methods**: Fifty-two enamel samples were randomly divided into four groups (n=13): CP16% –16% carbamide peroxide - Whiteness Perfect; HP6% - 6% hydrogen peroxide - White Class; HP35% - 35% hydrogen peroxide Whiteness HP Maxx; and Control - not bleached and kept in artificial saliva. For roughness analysis, average surface roughness (Ra) and flatness coefficient (Rku) parameters were used. The topography and permeability were examined by SEM. For permeability evaluation, the samples were immersed in a 50% silver nitrate solution and analyzed using a backscattered electron and secondary electron mode. **Results**: For the roughness (Ra) evaluation, Kruskal-Wallis and Wilcoxon Signed Ranks Test were used, showing an increase on the surface roughness in all bleached groups. The Rku parameter suggested changes on enamel integrity. The SEM micrographs indicated changes on enamel topography and different levels of silver nitrate penetration in the samples of the bleached groups. In the overall analysis, the bleaching agents promoted surface changes and higher silver nitrate penetration when compared to the control group. **Conclusions**: It may be concluded that different bleaching agents might alter the topography and roughness of enamel surface. Moreover, the higher infiltration of silver nitrate suggests an easier penetration path for the oxygen molecules into the dentin substrate.

Keywords: enamel, dentin, silver nitrate.

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Introduction

The change in tooth color is the result of a complex physical and chemical interaction between the tooth and the pigmentation factor[1]. Bleaching treatment for vital teeth is a conservative technique obtaining suitable results when compared to more invasive procedures used in aesthetic and cosmetic rehabilitation[2]. Usually bleaching gels contains carbamide peroxide (CP) or hydrogen peroxide (HP). Since CP dissociates into urea and HP, the action of CP is produced in the same way as bleaching agents based on HP[3].

Although their chemical action mechanism of bleaching agents is not entirely

understood, its known they must diffuse through the dental structure for the bleaching procedure to be effective[4]. HP has low molecular weight chain and is decomposed into H_2O and O^-, the later is a free radical associated with high permeability and diffusibility in the tooth structure[2,5]. O^- molecules attack the long-chained, dark-color macromolecules of pigments and split them into smaller, less colored and more diffusible molecules, which are easier to be removed from the structure, producing the bleaching effect[4,6].

The free radicals (O^-) released from HP permeates into the enamel subsurface through interprismatic regions and may reacts not only with pigmented organic molecules, but also with the organic enamel matrix. As the organic phase is mainly distributed in the inter-zones of inorganic structures, i.e. prisms, the removal of organic material increases the surface irregularity[7-8]. The diffusibility depends on factors such as the composition and concentration of the penetrating product, area, exposed surface location, exposure time, temperature and nature of the substrate to be penetrated[5]. A model was proposed to assess the penetration through dental hard tissues, including enamel, using silver nitrate[9]. This methodology may track the channels used by O^- to penetrate into the enamel structure.

Questions are still raised about the possible deleterious effects caused by products containing HP and CP. Studies have revealed mild to severe alterations[3,7,10-17]. while others have found no significant changes to the surface area with at-home or in-office bleaching techniques[18-25]. Although several studies have been focused on morphological changes on dental surfaces after bleaching, this seems to be one of first studies reporting on the jump-start approach using silver nitrate penetration to track and evaluate the possible channels of O^- penetration.

In this context, the aims of this study were to evaluate by profilometry and scanning electron microscopy (SEM) the effect of two gels for at-home use, CP at 16% and HP at 6%, and one gel for in-office use, HP at 35%, on the surface of bovine enamel, and to determine the correlation of these two variables with the ammoniacal silver nitrate penetration onto enamel surface. The hypothesis tested is that the use of at-home and in-office bleaching agents increases enamel roughness by changing its topography.

Material and methods

Enamel Specimens – Bleaching Procedures

Fifty-two freshly extracted bovine central incisors were cleaned and stored in a 0.2% aqueous thymol solution (Pharmacia Biopharma Ltda., Uberlândia, MG, Brazil) until use. Using a double-face diamond disc (KG Sorensen, Barueri, SP, Brazil) in a low-speed handpiece (Kavo, Biberach, Germany) under copious water spray, the root portion were cut and eliminated. Then, the coronal pulp tissue was removed and the pulp chamber filled with light-activated composite resin (Filtek Z250, 3M ESPE, St. Paul, MN, USA). The buccal surfaces were then flattened as parallel as possible without exposing dentin with 600-, 1200- and 1500-grit SiC paper (Norton, São Paulo, SP, Brazil), and sectioned in a high-precision cutting machine (Isomet 1000; Buehler, Lake Bluff, IL, USA) to obtain square-shaped samples (5 mm x 5 mm) of the middle third of the buccal surface. All samples were checked with a digital caliper and under 40× magnification to eliminate those with flaws, irregularities or dimensional alterations were eliminated. Enamel thickness was standardized to 1.5 mm, and an elastomeric matrix was used to control thickness and flow of the bleaching agent. The samples were embedded into cylindrical polystyrene molds (Cristal, Piracicaba, SP, Brazil) and polished with 6, 3, ½ and ¼ μm diamond grit (Arotec, São Paulo, SP, Brazil). The samples were randomly divided into 4 groups (n = 10): CP at 16% (CP16% - Whiteness Perfect, FGM, Joinville, SC, Brazil; HP at 6% (6% HP - White Class, FGM); HP at 35% (35% HP - Whiteness HP Maxx, FGM), which were treated following the manufacturers' instructions (Table 1 and 2). The control group was not bleached and was kept in artificial saliva. All samples were stored in artificial saliva for 14 days.

Table 1: Bleaching gel treatment for the at-home technique protocol.

Group	Number of applications	Exposure time	Frequency	Total exposure
CP16%	14	4 h	daily	56 h
HP6%	14	2 h	daily	28 h

An approximately 1-mm-thick bleaching gel layer was applied on enamel surface. The thickness was controlled using an elastomeric matrix. After each gel application, the samples were rinsed with deionized water and stored in artificial saliva at 37°C, which was renewed daily. In order to simulate a night-guard situation, HP6% and CP16% groups, samples were kept in plastic containers with small amount of artificial saliva, over the time of gel application. The 35% HP based product, used in in-office bleaching techniques, was activated by a diode-argon emitted light laser unit (Whitening Laser, DMC, São Carlos, SP, Brazil) for 3 min, placed 10 mm far from the surface. After the last session of each group, the samples were rinsed and stored in deionized water.

Surface Roughness Test

After the bleaching procedures, specimens were rinsed with the water spray and air-dried. Surface roughness was measured using a surface profilometer (SJ-301 Surface Roughness Tester – Mitutoyo, Tokyo, Japan). The surface

Table 2: Bleaching gel treatment for the in-office technique protocol.

Group	Clinical sessions	Frequency	Applications per session	Exposure time per application	Total time of exposure
HP35%	2	7 days	3	9 min	54 min

roughness was measured five times for each specimen, and the average values obtained were defined as Ra (arithmetic average height) and Rku (flattening coefficient) of each specimen. The measurements were made before and after the bleaching and control procedures.

The arithmetic average height parameter (Ra), also known as the centre line average (CLA), is the most universally used roughness parameter for general quality control/analysis. Rku, called the Kurtosis coefficient, is the fourth central moment of profile amplitude probability density function, measured over the assessment length. It describes the sharpness of the probability density of the profile. An area normally distributed is presented by Kurtosis equal to 3. A distribution of centrally distributed topography height is presented by Kurtosis more than 3, while well scattered distributions of height are presented by Kurtosis less than 3. Therefore, if Rku is less than three, the surface area presents few high peaks and few deep troughs; if Rku is more than three, the area presents many high peaks and many deep troughs. The Rku parameter is also used to differentiate areas that have different topographies, but have the same value of Ra.

Data from initial and final roughness (Ra) were subjected to normality Kolmogorov-Smirnov and Shapiro-Wilk tests, and the analysis showed that data were not normal. Therefore, Kruskal-Wallis test was used for comparison of roughness (Ra) among the groups in the first and last periods. The Wilcoxon Signed Ranks Test was used to compare initial and final Ra values in each group ($p < 0.05$).

SEM Analysis

Two samples were used for SEM analysis. After vacuum sputtering with gold (MED 010, Balzers Union, Balzers, Liechtenstein), the samples were analyzed under ×20,000 magnification (LEO 435 VP, LEO Electron Microscopy Ltd., Cambridge, UK). A representative sample of each group had all sides protected by nail polish, except for the enamel surface. The enamel blocks were then immersed into 50% ammoniacal silver nitrate (Aldrich Chemical Co., Milwaukee, WI, USA) solution for 24 h, and then transferred to a developer solution (Kodak Professional D-76 developer, Kodak Rochester, NY, USA). The samples remained in the developer solution under a "day-light" fluorescent lamp positioned 15 cm away from the surface of the liquid, during 8 h. After the silver nitrate crystals developing process, the samples were submitted to 5 one-hour deionized water baths, renewed at every bath. The blocks were then cut lengthwise with diamond disc (KG Sorensen) mounted on a low-speed handpiece (Kavo) under water spray and polished with 1200- and 2000-grit SiC papers. The samples were then sputtered with carbon under high-vacuum ambient (MED 010) and examined by SEM at ×150-400 magnification using the backscattering electron detector and also on the secondary electron mode.

Results

Surface roughness analysis showed no significant differences ($p = 0.333$) in the initial values of Ra among the groups (Table 3). After treatment, all bleached groups showed increased surface roughness, with difference between initial and final roughness values within each group $p = 0.001$ (Table 5), confirming the tested hypothesis. However, the HP6% group showed significantly higher Ra than the other groups (Table 4).

Tables 3 and 4 show the mean results and standard deviation. The statistical analysis revealed data distribution as non-normal and so the average values are also provided along the result of the statistical analysis.

The initial Rku readings for all groups was lower than three (<3); therefore the areas are presented with few high peaks and few deep troughs. At the end of treatment (final readings) Rku had values greater than three (>3) in all groups, except for the control group, showing that the area began to present many high peaks and many deep troughs, which suggests changes in enamel integrity induced by the mineral loss.

Non-uniform changes in enamel surfaces exposed to bleaching agents were detected in SEM analysis (Figure 1), while the SEM micrographs of the control group were flat. Such changes were also more evident in the group bleached with HP6% (Figure 1C), which had more acute pores, irregularities and depressions, and noticeable boundaries of enamel prisms.

The SEM micrographs of silver nitrate-infiltrated samples (Figure 2) revealed different penetration gradients. The qualitative analysis showed that there was silver nitrate penetration in all groups, but the penetration through the interprismatic region occurred mainly in the experimental groups. In the control group (Fig. 2: A.1, A.2 and A.3), the detected penetration was more evident through enamel cracks and microcracks (see arrows in Fig. 2). SEM micrographs of the HP6% bleached group suggests higher penetration when compared to HP35% samples, with the HP6% group showing

Table 3: Results of the Ra roughness parameter comparison among groups at initial Kruskal-Wallis test ($p < 0.05$).

Group	Initial Average ± Standard Deviation	Initial (Mean Rank)	Final Average ± Standard Deviation	Final (Mean Rank)
Saliva	0.080 ± 0.035	141.04 **A**	0.084 ± 0.023	102.87 **B**
HP6%	0.069 ± 0.023	124.83 **A**	0.124 ± 0.038	171.26 **A**
CP16%	0.065 ± 0.023	112.78 **A**	0.088 ± 0.049	97.30 **B**
HP35%	0.071 ± 0.037	118.69 **A**	0.102 ± 0.039	126.80 **B**

Similar letters indicate no statistically significant difference (p = 0.333)

Table 4: Results of the Ra roughness parameter comparison of each group in the initial *vs.* final periods, by the Wilcoxon Signed Ranks Test ($p < 0.05$).

Group	Initial	Final	p
Saliva	25.28 **A**	21.74 **A**	0.201
HP6%	6.50 **A**	25.63 **B**	0.001
CP16%	19.27 **A**	25.81 **B**	0.001
HP35%	16.00 **A**	26.20 **B**	0.001

Different letters indicate statistically significant difference in lines (p <0.05).

Table 5: Comparison of the Rku roughness parameter value in each group during the initial and final periods.

Group	Initial Average	Final Average
Saliva	2.91	2.95
HP6%	2.95	3.99
CP16%	2.95	3.28
HP35%	2.93	3.37

Rku < 3: few high peaks and deep troughs; Rku > 3: several high peaks and deep troughs

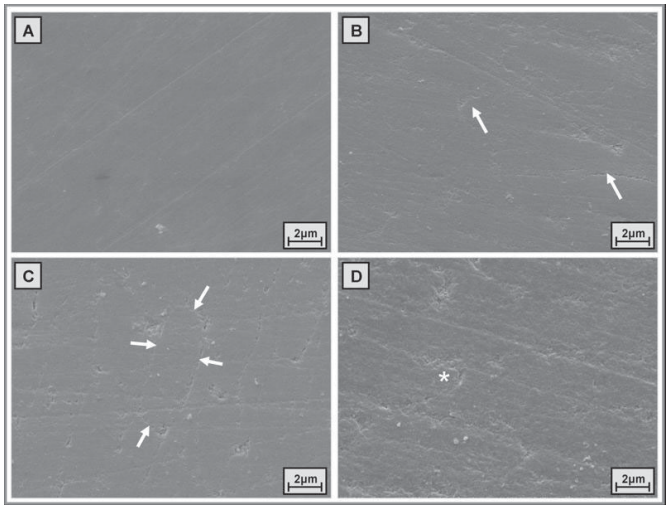

Fig. 1: Enamel surfaces after exposure to different bleaching agents. **A.** Non-bleached enamel surface morphology in the control group (C). Flat and unchanged surface; **B**. Exposed enamel surface to Whiteness Perfect (CP16%) - 16% Carbamide Peroxide. Discrete change can be observed, superficial and discontinued channels (arrows); **C**. Exposed enamel surface to White Class (HP6%) - 6% Hydrogen Peroxide. Obvious pores, irregularities and depressions, with clear evidence of enamel prisms boundaries (arrows); **D**. Exposed enamel surface to Whiteness HP Maxx (HP35%) – 35% Hydrogen Peroxide. Moderate porosity can be observed (asterisk). (All micrographs at 20.000x)

higher penetration among the prisms (see asterisks in Fig. 2). The CP16% group showed a distinct penetration pattern. Although low amount of silver nitrate was detected, there was still slight penetration through the prisms.

Discussion

Studies are controversial on the actual effects of bleaching agents onto dental enamel surface due to a variety of methodologies employed, use of different products and trademarks, concentration, pH, dosage, application protocols, criteria for analysis of results, and also the difficulty on linking surface changes to a bleaching agent in particular, due to enamel natural morphology and composition variation[16].

In this study, the tested hypothesis was confirmed. After bleaching treatment, all groups clearly showed an increase in surface roughness (Ra), in the same way as reported elsewhere[10-11,15]. The initial Rku parameter for all groups was lower than three, this confirms that the areas were presented with few high peaks and few deep troughs. At the end of the bleaching treatment, Rku was higher than three, showing

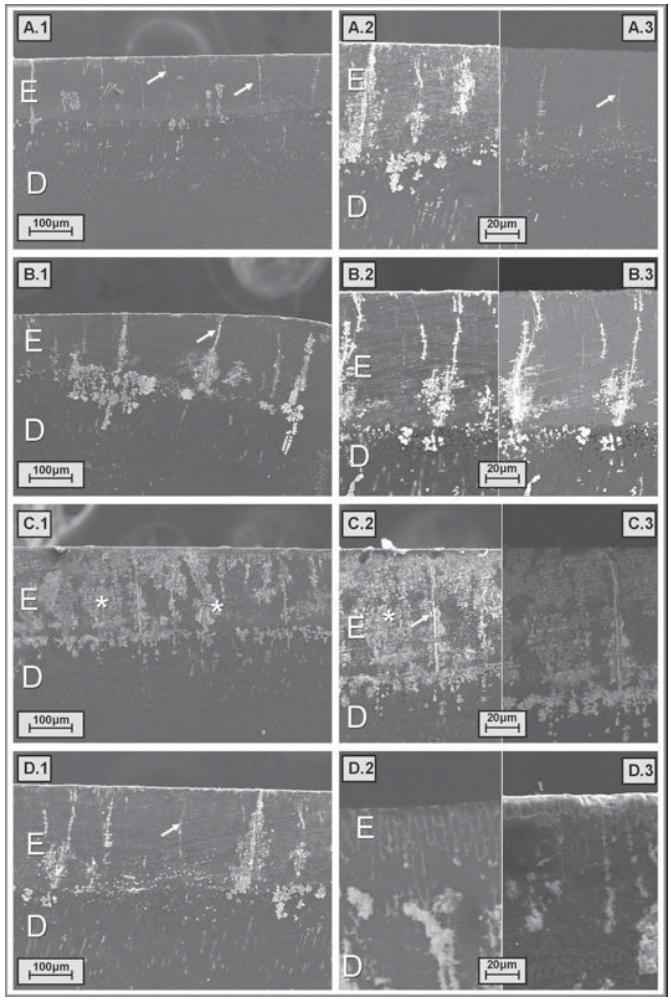

Fig. 2: Profile cuts of superficial enamel (E) and dentin (D), after the infiltration with silver nitrate (white and outshining points). **A.1:** Representative image of C (artificial saliva) group samples 150x infiltrated with silver nitrate; **A.2**: image with back-scattering electron detector. Image suggests penetration through the cracks and micro-cracks (see the narrows) present in enamel (400x); **A.3:** same image of A.2 but in secondary electron mode (400x); **B.1:** representative image of the CP16% samples Group 150 x infiltrated with silver nitrate; **B.2:** image with back-scattering electron detector. Image suggests slight penetration through prisms (400x); **B.3:** same image of B.2 but in secondary electron mode (400x); **C.1:** Representative image of HP6% group samples 150X infiltrated with silver nitrate; **C.2**: image with back-scattering electron detector. Image suggests great penetration through prisms (see asterisk) (400x); **C.3:** same image of B.2 but in secondary electron mode (400x); **D.1:** Representative image of the HP35% group samples 150x infiltrated with silver nitrate; **D.2**: image with back-scattering electron detector (400x); **D.3**: same image of B.2 but in secondary electron mode (400x). Image suggests moderate penetration through prisms (400x).

that the surface presented many high peaks and many deep troughs. The enamel surfaces exposed to bleaching agents analyzed by SEM showed changes, characterized as pores, irregularities and depressions. However they were not uniform, while the images of representative samples of the control group were presented as flat. However, only the group bleached with HP6% showed statistically higher values of Ra and also more evident changes in SEM analysis, when compared to the control group.

The use of ammoniacal silver nitrate to assess the rate of penetration in the tooth structures subjected to treatment with bleaching agents has been reported by Iwamoto et al.[9].

The fact that these authors have not found enamel penetration may be related to the shorter exposure time to silver nitrate, 1 h. In this study, after 24 h of immersion[26-29], two distinct paths can be noticed when the silver nitrate particles penetrated through the enamel and reached dentin: through the prism and through the microcracks typical of the enamel structure[30]. The fact that silver nitrate penetrated the area suggests an easy oxygen penetration, since the atomic weight of oxygen is approximately 7 times less than silver.

The analysis of silver nitrate penetration in the control group when correlated with the findings of roughness and topography of the area examined by SEM, suggests that the smaller the surface change, the lower the enamel penetration. A possible explanation for this would be the lower surface energy of this group compared to the other bleached groups. This reduction in energy surface could be responsible for the lower moistening of the enamel and consequently lower action of the bleaching gel[31]. The opposite can also be hypothesized for the HP6% group, which had significant changes in enamel topography and roughness, coinciding with the highest nitrate penetration.

Daily applications (30 min during 14 days) of HP gels with low concentration were measured by Pinto et al.[15], who found no significant increase in roughness when compared to the control group stored in artificial saliva. A similar finding was obtained in the present study with the HP6% group, with daily application of the gel of 2 h, which is the maximum time recommended by the manufacturer. The group bleached with HP at 35%, despite being about 6 times more concentrated showed lower roughness when compared to HP6%. However, the total period of application was 54 min within 14 days, while in the HP6% group it was 28 h. The period of direct contact of the bleaching agent in the tray with the tooth surface makes the enamel more prone to suffer topographic changes. This situation was not observed with the high concentration of agents used in in-office techniques, which accelerates the peroxide decomposition due to activation by light source or heat[32-33]. According to Bitter and Sanders[34], it is believed that peroxide concentration and the contact time of the agent with the tooth surface, are directly proportional to the changes caused by the agent in the bleached substrate.

The group bleached with CP at 16%, which is composed of approximately 5.7% HP, induced minor changes in enamel surface, after daily applications for 4 h. This result is conflicting with the result of the HP6% group, which showed the greatest superficial change. This finding could be justified by the buffering capacity of saliva[35-36] and the presence of urea, which is a CP degradation product. Urea is degraded into ammonia and carbon dioxide; the ammonia reacts with moisture and produces ammonia dioxide that has the ability to raise the pH[3]. In addition, home-use bleaching gels based on HP, do not have urea as a decomposition product and so they do not raise the pH leading to non critical levels of demineralization.

The oxidative process and the pH of bleaching agent are regarded as the main causes of the adverse effect of dental enamel after the bleaching treatment. The capacity of the oxidative process to create irregularities on the surface of bleached enamel is questionable as the peroxide activation nature and the interaction with the various bleaching gel components need to be determined[37]. According Price et al.[38], it is unclear whether the pH of products containing CP or HP undergoes similar changes in the oral cavity or whether these changes can affect the dental tissues during the bleaching process and the intraoral temperature can affect the pH. However, regarding the effects of acidic and basic solutions, it is important to consider the time of exposure and frequency of use of the product.

Although the artificial saliva used in laboratory studies can present some remineralization capacity[39], it is important to note that the dynamics of saliva/enamel interaction is a difficult factor to fully replicate in laboratory research. It has been demonstrated that the saliva remineralization effect may prevent the demineralization effect on bleaching treatment of human enamel *in situ*, and that the amount of calcium loss was 2.5 times higher *in vitro* than *in situ*[40]. Although this does not invalidate the results of this study, it must be observed and analyzed carefully. Clinical studies evaluating the propriety and mechanisms of action of bleaching agents are also necessary to guide professionals in the selection of the bleaching technique that presents the least dielectric effect onto enamel surface[40].

According to the employed methodology and the obtained results, it may be concluded that:

• various bleaching agents promote superficial changes in enamel structure surface;

• the fact that silver nitrate has permeated the area suggests an easy penetration by the oxygen produced by bleaching agent decomposition, and also suggests a subsuperficial damage to enamel tissue.

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