

***In vitro* Evaluation of Morphological Integrity of Dental Enamel Exposed to Carbamide Peroxide-based Bleaching Agent**

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This article presents the effect of the bleaching above the enamel of the teeth using three fundamental approaches for bleaching non vital teeth. Our purpose is to assess the effects of the carbamide peroxide on the structure of the tooth enamel at different concentrations and to check if the destructive effects are important at low concentrations. In order to assess the morphological and topographical modifications of the whitening treatment, the three samples (incisor, premolar, molar) were analyzed with a Scanning Electron Microscope, type Quanta 200 (FEI Company). More detailed morphological aspects of the samples before and after the whitening treatment were analysed using a Scanning Probe Microscope (Solver PRO-M, NTMDT, Russia) and various tridimensional statistical parameters were calculated from the AFM measurements. Significantly higher 3D roughness parameters indicate that the surfaces were more irregular, because the demineralization process of the enamel, exhibiting numerous and deeper peaks and valleys.

Keywords: bleaching teeth, SEM, AFM, carbamide peroxide

Tooth whitening is a method used for the treatment of the dental discoloration by applying some substances that contain hydrogen peroxide or its precursor, carbamide peroxide, having a concentration ranging between 6% - 40%, depending on the application method. There are three fundamental approaches for bleaching vital teeth: in-office or power bleaching with 25-40% hydrogen peroxide, at-home, or dentist-supervised night-guard bleaching with 10-20% carbamide peroxide, and over-the-counter products with a low concentration of peroxide, 3-6%, self applied to the teeth via gum shields, strips, dentifrices [1, 2]. Commonly known local risks associated with tooth bleaching include tooth sensitivity, gingival irritation and adverse effects on enamel and restorative materials [3-5]. The mechanism of the tooth bleaching is based on the hydrogen peroxide that penetrates the tooth, produces free radicals and interacts with pigment molecules, oxidizing them and changing the configuration and size [6-8]. Although the manufacturers consider that the application of the hydrogen peroxide gel does not cause major side effects on the enamel's morphology, *in vitro* researches have shown the existence of some structural modifications: surface morphological changes, alteration of surface microhardness and mineral loss, chemical composition, when it using 30% hydrogen peroxide and more [9, 10]. On the contrary, other studies have reported no negative effects on surface microhardness when it using 10% carbamide peroxide and it is considered safe and effective [11, 12].

The aim of our study is the *in vitro* analysis of the morphological changes through Scanning Electron Microscope (SEM) and Atomic Force Microscopy (AFM). In dentistry, SEM method is also used for testing the morphological structure of other materials, like, dental alloys and materials containing acrylic products [13]. Our purpose is to assess the effects of the carbamide peroxide on the structure of the tooth enamel at different concentrations and to check if the destructive effects are important at low concentrations.

More detailed morphological aspects of the samples before and after the whitening treatment were analysed using an Atomic Force Microscopy that is type of Scanning

Probe Microscopy (SPM). The AFM has three major abilities: force measurement, imaging, and manipulation. AFM image is a simulated image based on the height of each point of the surface and, in fact, each point (x, y) of the surface has a height $h(x, y)$ [14].

Experimental part

We used sound teeth extracted for orthodontic and periodontal reason (incisor, premolar and molar). After the surfaces was cleaned under high pressure water to remove the white material, the teeth were stored in 0.1% thymol solution at 4°C until the preparation for testing. The teeth were treated with 10% (incisive), 16% (premolar) and 35% (molar) carbamide peroxide (44% Teeth Whitening Gel, WGS44-10, United States) solutions, respectively. The pH of the carbamide peroxide solutions was 7.4 (measured in laboratory). The bleaching treatments were performed for each tooth in two sessions, over a period of three months, at room temperature in closed dishes for 30 min/day over three months. The total treatment time was 90 min.

Measurements

The surface images were obtained with a Solver PRO-M scanning probe microscope (NT-MDT, Russia) in AFM configuration. Rectangular silicon cantilevers NSG10 (NT-MDT, Russia) with tips of high aspect ratio were used. All images were acquired in air, at room temperature (23 °C), in tapping mode, and at a scanning frequency of 1.56 Hz. The scan length ranged between 5 and 20 μm .

Each tooth was mounted on an aluminum stub for outer enamel structural defects examination by SEM (FEI Quanta 200, Eindhoven, the Netherlands) operating at 20 kV in low-vacuum mode for secondary electron imaging.

Results and discussions

Scanning Electron Microscopy (SEM)

As shown in figure 1, the surface of the teeth is different from one another. The incisor's surface is rougher and has more sediments than the others, the premolar's surface is mostly smooth with scattered sediments and the molar's surface presents traces from mastication.

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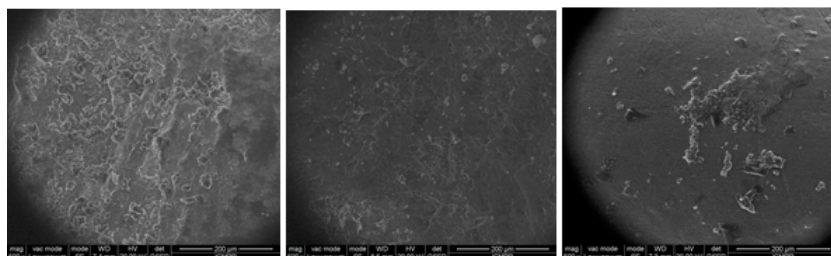


Fig. 1. Initial SEM micrographs of incisor, molar and premolar

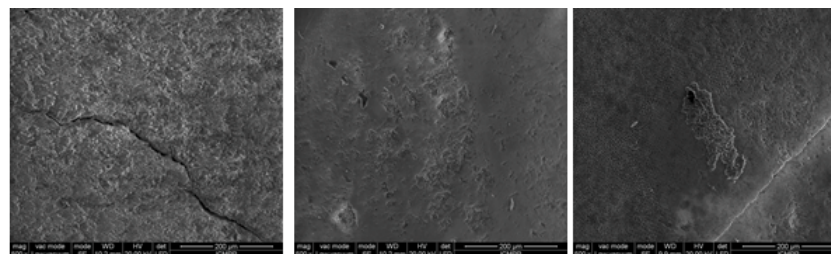


Fig. 2. SEM micrographs of incisor, molar and premolar after the first whitening treatment

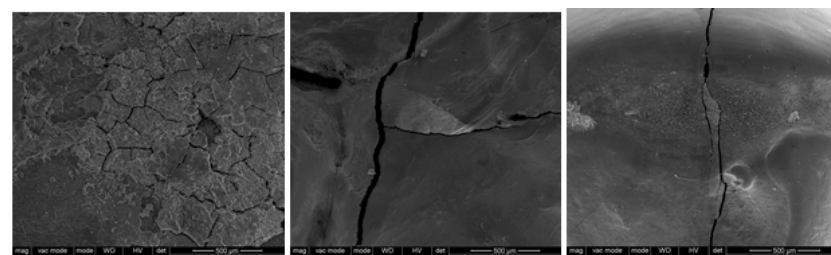


Fig. 3. SEM micrographs of incisor, molar and premolar after the second whitening treatment

These micrographs show that there are morphological modifications of the surface after applying the lowest concentration of carbamide peroxide. In the case of the incisor, the initial sediments have been replaced by enamel fragments due to the demineralization of the incisor's surface. Also, due to the same process, narrow fractures appear on the surface. On the surface of the premolar, narrow and straight fractures appear, whilst the enamel is demineralized more severely, in certain regions. The molar's surface presents scattered demineralization holes in the enamel that might be due to the wider surface of the tooth. In figure 2 are shown the micrographs obtained after the first whitening treatment with 10% (incisor), 16% (premolar) and 35% (molar) carbamide peroxide solutions.

Figure 3 shows micrographs of the incisor, molar and premolar after the last bleaching exposure. The surface of the teeth is clearly different and more damaged compared to the initial micrographs. The incisor's enamel was severely deteriorated, hence the presence of microscopic fractures on the entire surface. In addition, there are areas where the enamel was completely destroyed and revealed the dentine underneath. In the case of the premolar, the fractures widened and deepened, whilst the enamel deteriorated in some regions, also revealing the dentine. The molar's surface, although rather smooth, has increased holes (up to 400 μm) and fractures. As shown in the micrograph, the fractures vary in depth and width, depending on the topography of the initial surface.

It is clear that the damage of the enamel increases with the concentration of carbamide peroxide and that the morphological changes differ from one tooth to another.

The different modifications of the teeth may be due to the size and mechanical properties of each one, hence the more severe degradation of the enamel of the incisor compared to the molar.

Atomic Force Microscopy (AFM)

More detailed morphological aspects of the samples before and after the whitening treatment were analysed using a SPM (Solver PRO-M, NTMDT, Russia) and a commercially available NSG10 cantilever (Solver PRO-M, NTMDT, Russia) with the resonant frequency of 297 kHz. Different squares of various side were scanned in the semi-contact mode, but the morphological features were easily observed when the scan length of 5 μm was used. The height AFM images collected in air, at room temperature (23 $^{\circ}\text{C}$) and analysed using the software Nova v.1.26.0.1443 for Solver were clearly obtained for molar and premolar samples (figs. 4 and 5). Unfortunately the incisor's surface was very rough, especially after the whitening treatment, the maximum peak-to-valley distance (S_z) being higher than the limit allowed by our device. Thus, the samples in question were only the molar and premolar.

The surface topography of the molar and premolar samples, before and after the first and the second whitening treatment was evaluated by means of height AFM images, presented in figures 4 and 5, respectively. Using a specialized and standardized software, various tridimensional statistical parameters were calculated from the AFM measurements, among them being the maximum peak-to-valley distance (S_z) which is the mean distance from the highest peak to the lowest valley in the sampling

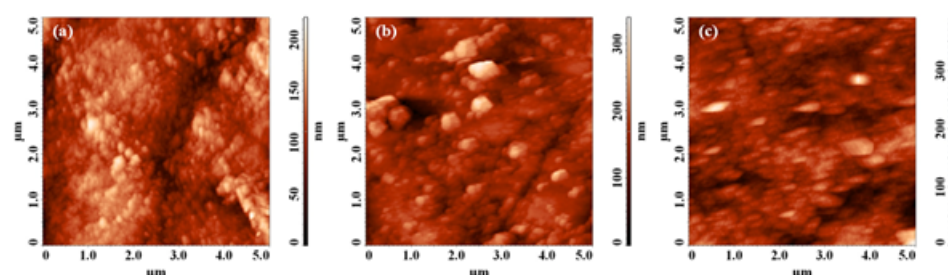


Fig. 4. 2D topographical images of the molar surface before (a) and after the first (b) and the second whitening treatment (c).

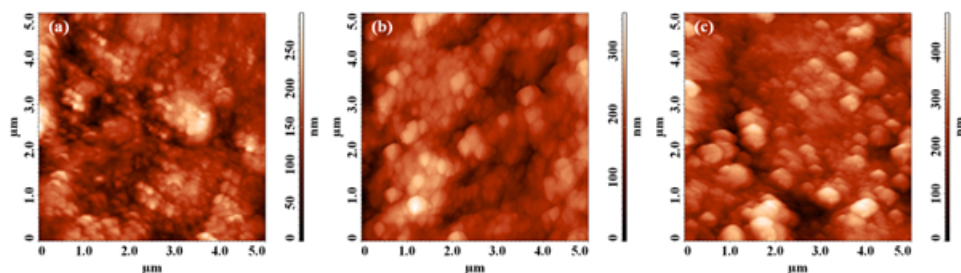


Fig. 5. 2D topographical images of the premolar surface before (a) and after the first (b) and the second whitening treatment (c).

Table 1

CHANGES SZ, ROOT MEAN SQ, AND DEVELOPED SDR OF MOLAR AND PREMOLAR ENAMEL BEFORE AND AFTER THE FIRST (W1) AND THE SECOND WHITENING TREATMENT (W2)

Sample	3D surface roughness parameters calculated on 5x5μm ²		
	Sz (nm)	Sq (nm)	Sdr (%)
molar	221.6±8.8	30.7±1.2	8.355±0.334
molar w1	338.9±13.5	36.6±1.5	14.872±0.595
molar w2	389.9±15.6	45.4±1.8	18.125±0.725
premolar	300.5±12.1	40.2±1.6	11.940±0.478
premolar w1	359.1±14.3	45.3±1.8	18.526±0.741
premolar w2	482.3±19.3	63.6±2.5	26.167±1.047

area, the root mean square roughness (Sq) defined as the root mean square value of the surface departures within the sampling area and a quantification of the shape-size complexity, and the developed surface area ratio (Sdr) which is the percentage of additional surface area contributed by the texture as compared to an ideal plane the size of the measurement region. All these parameters shown in table 1, mediated from five different measurements, describe the relief structure quality and the complexity of the morphological features.

Initially, according to figures 4 and 5 and table 1, the enamel of the pristine molar sample was smoother compared to the enamel of the pristine premolar sample. Further, depending on the bleaching protocol, different effects on dental enamel structure and topography were observed. First the morphology for both samples was strongly influenced by the whitening process with 16% and 35% carbamide peroxide solutions, respectively (figs. 4 (b) and 5 (b)). Significantly higher 3D roughness parameters obtained for these samples compared to the unmodified ones indicate that the surfaces were more irregular, due to the demineralization process of the enamel, exhibiting numerous and deeper peaks and valleys (the values for Sz). The application of the second whitening treatment with 16% (premolar) and 35% (molar) carbamide peroxide solutions revealed more intense morphological changes on enamel surfaces influenced by previously created porosity, allowing the penetration of the high-concentrated bleaching agent on deep enamel. The significant deep alterations of the dental structure were reflected also by the increased values of the Sz, Sq and Sdr.

Conclusions

Summarizing the results of the effect of three bleaching concentrations (carbamide peroxide solutions) and their application on the morphological modifications of enamel, the following conclusions can be gained: The investigation

of carbamide peroxide bleaching agent in three different concentrations, two times over a period of three months, showed that surface morphology of the enamel are affected, causing enamel erosion, as SEM and AFM techniques have revealed. According to the results of this study it is recommended to perform tooth whitening using only low concentration of carbamide peroxide (10%), and shorten treatment time (<30 min) to reduce the possible destructions in enamel structure.

References

- ALQAHTANI, M.Q. Saudi Dent J., **26**, 2014, p. 33.
- TANAKA, R., SHIBATA, Y., MANABE, A., MIYAZAKI, T., Oral Biology, **55**, 2010, p. 300.
- LI, Y., GREENWALL, L., British Dent. J., **215**, 2013, p. 29.
- REIS, A., KOSSATZ, S., MARTINS, G., LOGUERCI, A., Oper. Dent., **38**, 2013, p. 386.
- DAHL, J.L., PALLESSEN, U., Crit. Rev. Oral Biol. Med., **14**, 2003, p. 292.
- EIMAR, H., SICILIANO, R., ABDALLAH, M.N., NADER, S.A., AMIN, W.M., MARTINEZ, P.P., J. Dent., **40**, 2012, p. 25.
- SHI, X.C., MA, H., ZHOU, J.L., LI, W., Int. J. Oral Sci., **4**, 2012, p. 208.
- BUKHARI, C., LAZAR, L., MIHALY, T., FAZAKAS, Z., VLASA, A., SABAU, R., BERESCU, L., PETCU, B., KOVACS, M., Rev. Chim.(Bucharest), **67**, no. 5, 2016, p. 939.
- BEN-AMAR, A., LIEBERMAN, R., GORFIL, C., BERNSTEIN, Y., Am. J. Dent., **8**, 1995, p.29.
- TEZEL, H., ERTAS, O.S., OZATA, F., DALGAR, H., KORKUT, Z.O., Quintessence Int. **38**, 2007, p. 339.
- LOPES, G.C., BONISSONI, L., BARATIERI, L.N., VIEIRA L.C., MONTEIRO, S.Jr., J. Esthet. Restor. Dent., **14**, 2002, p. 24.
- PINTO, C.F., PAES, LEME, A.F., CAVALLI, V., GIANNINI, M., Braz. Dent. J., **20**, 2009, 48-53.
- LUPU, I.C., AGOP-FORNA, D., SANDU, I.G., MOCANU, C., FORNA, N.C., Rev. Chim. (Bucharest), **66**, no. 6, 2015, p. 808.
- SHARIFI-VAAND, A., MAHJANI, M.G., MOSHREFI, R., JAFARIAN, M., Vacuum, **114**, 2015, p. 1

Manuscript received: 12.02.2016