Effects of Fluoride or Nanohydroxiapatite on Roughness and Gloss of Bleached Teeth

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ABSTRACT Objectives: The aim of this study was to describe roughness and gloss alterations of enamel after treatment with 38% hydrogen peroxide (HP) and after polishing with 2% neutral sodium fluoride (SF) or a dental tooth paste containing nanohydroxiapatite particles (nHA) using power spectral density (PSD) description, roughness parameters (Ra, RMS, and *Z* range) and gloss analysis. Methods: An atomic force microscope (AFM) and a spectrophotometer were used to analyze eighteen specimens of upper incisors. After initial analyses, all specimens were bleached with 38% HP for 135 min. The specimens were analyzed after bleaching. Nine specimens were polished with SF (Group Fluor) and the other nine specimens were polished with nHA (Group nHA), then all specimens were analyzed after polishing. Roughness and gloss were analyzed with ANOVA and Tukey's *t*-test. Results: No statistical difference was found for Ra and RMS among initial, after bleaching and after polishing in both groups. For *Z* range, Group nHA showed a significant decrease after polishing. Bleaching with 38% HP did not increase the PSD in the spatial frequency of the visible light spectrum range in both groups. After polishing, nHA group showed a decrease in PSD for all morphological wavelengths. Gloss did not show statistical difference after bleaching in both groups. Gloss showed significant increase after polishing with nHA. Significance: bleaching treatment with 38% HP didn't alter enamel surface roughness or gloss. PSD analyses were very suitable to identifying the morphological changes on the surfaces. *Microsc. Res. Tech. 74:1069–1075, 2011.* © 2011 Wiley Periodicals, Inc.

INTRODUCTION

Home and in-office bleaching regimes are often classified as cosmetics, and therefore, there is no detailed requirement to ensure material safety or efficacy in dental treatment. Generally, more highly concentrated peroxides have been applied in the office setting, as the excesses and timing of peroxide application can be strictly managed. The advantages of in-office bleaching over home bleaching include dentist control, avoidance of soft-tissue exposure and reduced total treatment time (Tanaka et al., 2010).

With regard to potential adverse effects, hydrogen peroxide bleaching is not associated with macroscopically or clinically visible damage to dental hard tissue; however, concerns have been expressed regarding surface alterations in enamel topography. These surface alterations may be related to color and gloss alterations. The perception of color is a psychophysical phenomenon, and the measurement of color must be defined in such a way that the results correlate accurately with what the visual sensation of color is to a normal human observer.

Hydrogen peroxide can result in a decrease of the superficial microhardness of enamel (Zantner et al., 2007), to the extent that it is necessary to apply a low-concentrate fluoride to restore the initial level of hardness (Setien et al., 2009). This effect has been reported even about low-concentrate hydrogen peroxide products. Also, it has been shown that this bleaching product significantly reduces the calcium concentration of enamel surfaces (Tezel et al., 2007). The regular clinical application of fluoride gel treatments was found to be sufficiently effective to avoid loss of enamel microhardness during bleaching (Leandro et al., 2008; Lewinstein et al., 2004; Wiegand et al., 2007). Fluoride might also contribute to the repair of the microstructural defects of bleached enamel.

Recently hydroxyapatite materials have been discovered to have remineralizing effects on the altered enamel surface, helping in the recovery of teeth microfractures increasing teeth brightness and whiteness. It is reported that the remineralization effect is increased if the particle size of hydroxyapatite is reduced down to the nanometric range. Indeed, the interaction of nanoparticles with dentine and enamel is more effective, due to the increased surface to volume ratio (Generosi et al., 2010).

In dentistry, atomic force microscope (AFM) has been used successfully to study the surface topography of enamel and dentin. It has also been used in a study

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confirming a decrease in physical properties of enamel and dentin after bleaching with 35% hydrogen peroxide (Hairul Nizam et al., 2005).

The most popular parameter characterizing the morphology of surfaces are Ra, RMS, and *Z* range that represent the roughness of the surface around its mean values. However this statistical description, though simple and reliable, makes no distinction between peaks and valleys and does not account for the lateral distribution of surface features. A more complete description is provided by the Power Spectral Density (PSD) of the surface topography, which performs a decomposition of the surface profile into its spatial wavelengths and allows comparison of roughness measurements over different spatial frequency ranges. Thereby scattering theories predict that in the smooth-surface limit the angular distribution of light scattered from a surface fits the PSD of the surface topography (Gavrila et al., 2007).

From the PSD curve of the surface roughness, characteristic and valuable topographic parameters, referred to as the fractal dimension and the overall magnitude of the micro-roughness profile can be readily derived (El Feninat et al., 2001).

Considering that gloss is an optical property which is based on the interaction of light with physical characteristics of a surface, changes on surface morphological wavelengths on the same order of magnitude of the visible light wavelengths are responsible for visual changes, like changes in gloss (Pedreira de Freitas et al., 2010).

No conclusive data are available on the changes in surface morphology after bleaching treatment and its relation with gloss alteration. New toothpastes stimulated the scientific discussion on possible novel applications of nanotechnologies in pharmaceutical and cosmetic products: on their safety, efficiency, and action mechanisms. It should be noted, however, that the literature available is extremely scarce.

The aim of this study was to describe the topography alterations of enamel after treatment with 38% hydrogen peroxide and after polishing with 2% neutral sodium fluoride gel or a dental tooth paste containing nanohydroxiapatite particles (Nano<mHAP> agent) using a PSD description and gloss analysis.

MATERIALS AND METHODS

Eighteen intact, noncarious human maxillary incisors were used. The teeth were cleaned and stored in thymolized saline at 4°C for 48 h. Approval by the Ethics Committee of the School of Dentistry, University of São Paulo (FOUSP, protocol 142/2009) was obtained.

The teeth were sectioned with double face diamond discs (Discoflex, KG Sorensen, Brazil) to separate crown and root. Pulp cavities were cleaned and A3 composite resin (Z250, 3M ESPE, USA) was inserted to fill the chamber. All specimens were cleaned with aqueous slurry of pumice and anionic detergent, according with clinical procedures. In order to preserve the natural structure of enamel, no other agents were used to clean the teeth. During the experimental intervals, teeth were stored in Hank's balanced salts solution (HBSS) according to Habelitz et al. (2002) at 4°C.

First, a predetermined area from enamel surface of each specimen was imaged with an atomic force microscope using intermittent contact mode. All specimens were analyzed at room temperature, in air, in their dehydrated state by a Nanoscope IIIa (Veeco - Digital Instruments, Santa Barbara, CA). Etched silicon probes cantilevers were used (nominal resonant frequency $\omega \sim 250$ kHz, nominal spring constant, $k \sim 40$ N/m, and nominal probe tip end radius $\sim$10 nm). Images were obtained with a slow scan rate (below 1 Hz), which is the number of trace and retrace scan lines performed per second, and a resolution of 512 × 512 pixels per image was chosen.

Images with 10 μm × 10 μm were taken with the AFM software Nanoscope IIIA (Software version 5.12r3, Veeco - Digital Instruments, Santa Barbara, CA) of each specimen and the whole image was used for surface characterization. To quantify micro-roughness, the following methodology was adopted: first, conventional statistical roughness parameters, including Ra that is the arithmetic mean deviation of the surface; RMS that is the root-mean-square deviation of the surface; and *Z* range that defines the height between the highest peak and the deepest valley were determined. Then, to further characterize the surface morphology a spectral analysis of the AFM data were made using a fast Fourier transform (FFT) program. From the PSD curve of the surface roughness, characteristic, and valuable topographic parameters, referred to as the fractal dimension and the overall magnitude of the micro-roughness profile can be readily derived; this makes AFM a powerful tool that provides quantitative information not only on the height deviation of roughness profile, but also on its lateral distribution (the spatial extent of the height variations in the roughness profile), independent of the scan-frame size.

Gloss was measured using a spectrophotometer (CM-2600d, Konica Minolta Sensing Inc., Japan) with a 3 mm aperture. A custom-made polytetrafluoroethylene mold was placed over the specimen during measurements to enable accurate specimen positioning and eliminate the influence of the overhead light.

After the "initial" measurements were made, the specimens were exposed to a 38% hydrogen peroxide bleaching agent (Opalescence Boost™ PF- Ultradent Product Inc., South Jordan, UT) with pH ~ 7,0 (according to manufacturer's information). The bleaching product was handled according to the manufacturer's instructions and was applied as a 1 mm thick layer on enamel surface of each specimen for 15 min, with no light source application. After this period, gel was removed with gauze and deionized water. This procedure was repeated nine times for each specimen, totalizing 135 min of bleaching treatment. At the end of bleaching process, specimens were gently washed and sonicated in deionized water for 10 min to remove residual particles.

After bleaching procedure, all specimens were submitted to AFM and gloss analyses with the same parameters described before, to obtain surface information and images after bleaching.

Nine specimens were polished with 2% neutral sodium fluoride gel (Group Fluor). The 0.5g of fluoride gel remained on specimen's labial surface for 4 min then, buccal surface of each specimen was polished by

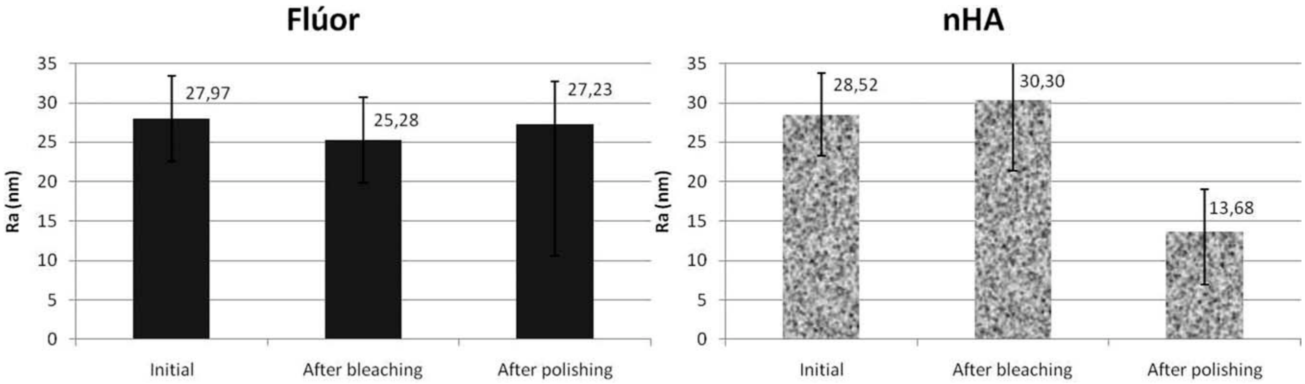

Fig. 1. Ra mean surface roughness (in nm) of Fluor and nHA Groups before bleaching (initial), after bleaching, and after polishing. Bars represent standard deviation interval. No statistical difference was found for group Fluor ($P = 0.92$) nor for group nHA ($P = 0.059$).

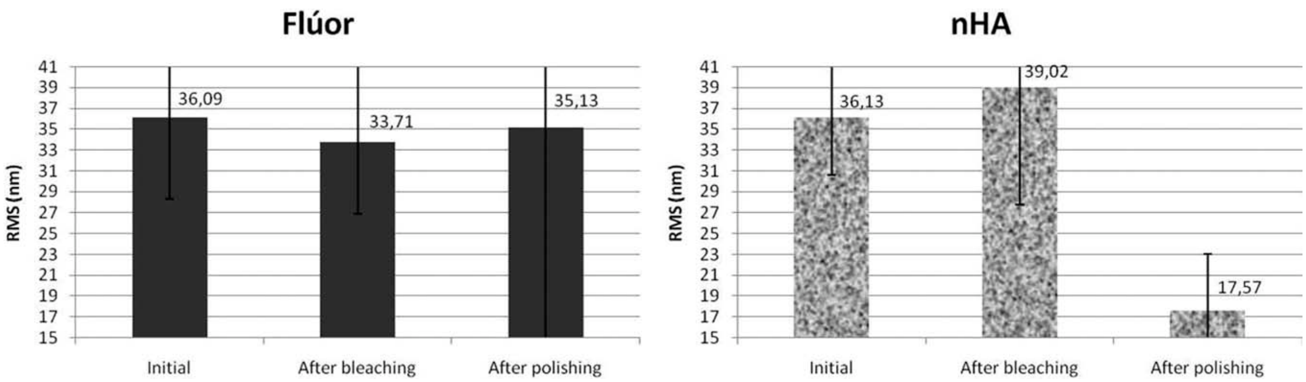

Fig. 2. RMS mean surface roughness (in nm) of Fluor and nHA Groups before bleaching (initial), after bleaching and after polishing. Bars represent standard deviation interval. No statistical difference was found for group Fluor ($P = 0.96$) nor for group nHA ($P = 0.051$).

a felt disc (FGM, Brasil) in a handpiece (Dabi-Atlante, Ribeirão Preto, Brasil) for 1 min with the gel on it. The specimens were cleaned with running tap water and sonicated in deionized water for 10 min. The other nine specimens were polished with a dental tooth paste containing nano-sized hydroxyapatite (Nano<mHAP> agent) (Apagard® Premio, Sangi, Japan) (Group nHA). A constant amount of 0.2 cm^3 of dental tooth paste remained on each specimen's labial surface for 4 min then, buccal surface of each specimen was polished by a felt disc in a handpiece for 1 min. The specimens were cleaned with running tap water and sonicated in deionized water for 10 min.

After the polishing procedure, all specimens were submitted to AFM and spectrophotometer again, to determine roughness and gloss after polishing.

Each specimen was used as its own control, thus reducing the effects of natural variation of enamel on the results of the experiment.

The Fourier transform calculations were made using the WSxM software. All subsequent numerical manipulations were done using Origin software (Northampton, MA). The average of the PSD of all the lines profiles in each of the images was used in order to improve the data analysis.

The results of Ra, RMS, Z range and gloss were analyzed by two-way analysis of variance ANOVA ($P < 0.05$) and Tukey's t-test ($\alpha < 0.05$).

RESULTS

For Ra and RMS no significant differences were found between all experimental procedures in both groups as it can be seen in Figures 1 and 2, respectively. Results demonstrated that the surface roughness of enamel did not increase after bleaching with 38% hydrogen peroxide because no significant differences in all roughness parameters were revealed when compared initial to after-bleaching. For Ra and RMS, none of the groups revealed statistical difference in roughness after polishing (Figs. 1 and 2). For Z range, nHA Group showed a significant decrease after polishing ($P = 0{,}033$) - Figure 3.

After 135 min of treatment with 38% hydrogen peroxide, the AFM images showed no alterations compared with the untreated surface images (initial) in both groups (Fig. 4).

AFM view images of the polished surfaces of groups Fluor and nHA are also displayed in Figures 4. It is possible to notice a slight decrease in roughness after polishing in both groups, but only nHA Group showed

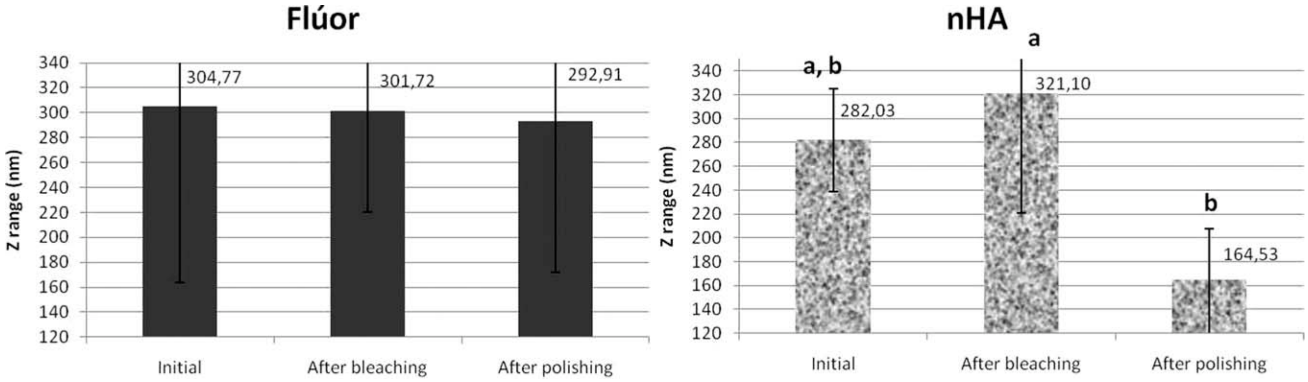

Fig. 3. Z range mean surface roughness (in nm) of Fluor and nHA Groups before bleaching (initial), after bleaching and after polishing. Bars represent standard deviation interval. No statistical difference was found for group Fluor (P = 0.98). For nHA Group, ANOVA showed significant difference (P = 0.033). Tukey's t-test is represented by the lowercases in the graphic.

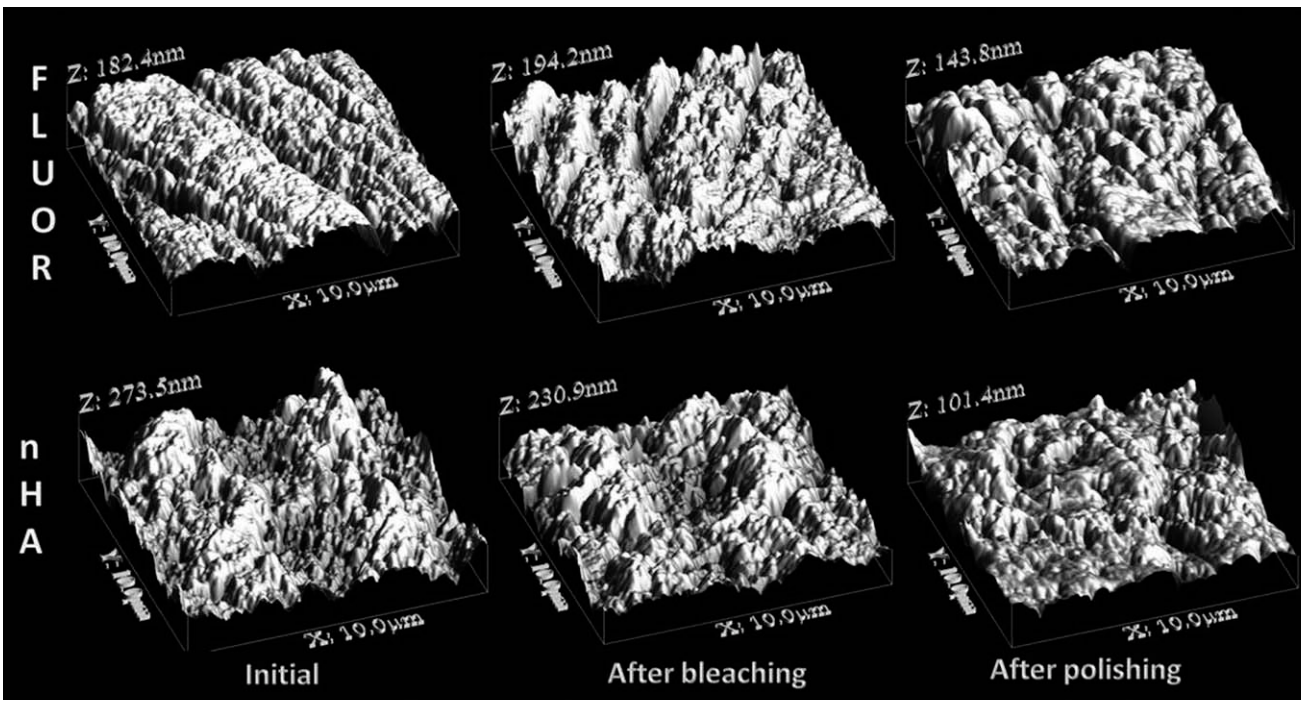

Fig. 4. AFM images of a Fluor group specimen and a nHA group specimen before bleaching (initial), after bleaching and after polishing.

significant decrease in the height between the highest peak and the deepest valley. In nHA group, the sides and the base of the grooves became more regular after polishing.

Figure 5 shows the PSD of the specimens before and after bleaching process and after polishing procedure in groups Fluor and nHA respectively. It is possible to notice that the 38% hydrogen peroxide treatment did not increase the PSD in the spatial frequency of the visible light spectrum range (380–750 nm) in both groups. After polishing, nHA group showed a decrease in PSD for all morphological wavelengths in this range.

Figure 6 plots the mean gloss values for the three experimental times of Fluor and nHA groups. No statistical significant difference was found among initial, after bleaching and after polishing procedures (P = 0.431) in Fluor group.

In nHA group, gloss showed significant increase after polishing procedure (P = 0.001). The result of the posthoc paired t-test is represented by lower cases in the graphic.

DISCUSSION

One of the aims of this study was to assess clinically relevant micromorphologic alterations of enamel surfaces after bleaching procedures. The results showed no statistically significant increase in Ra, RMS and Z range after bleaching agent exposure. Observing the images obtained with the AFM, it cannot be affirmed

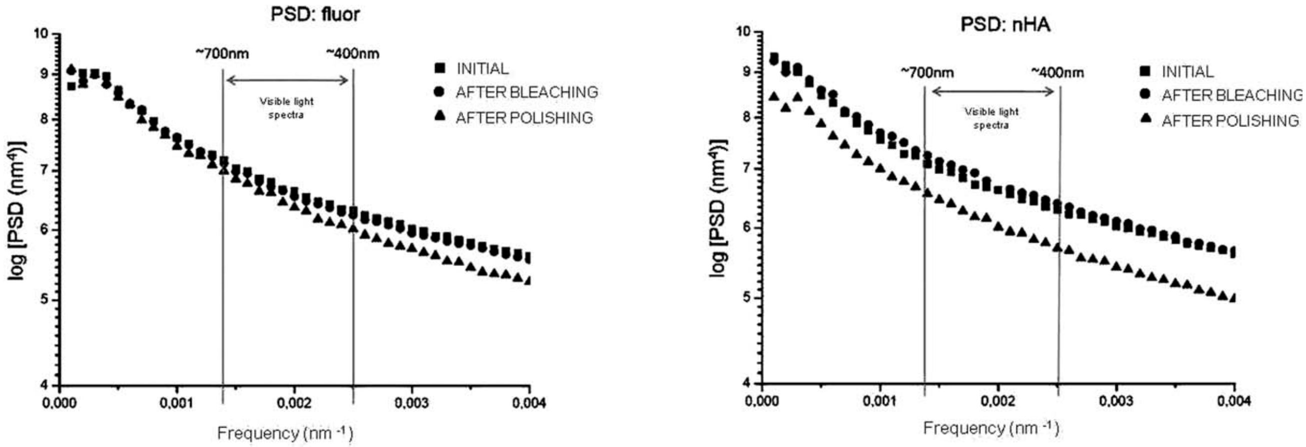

Fig. 5. Power Spectral Density of Fluor group and nHA group before bleaching (initial), after bleaching and after polishing.

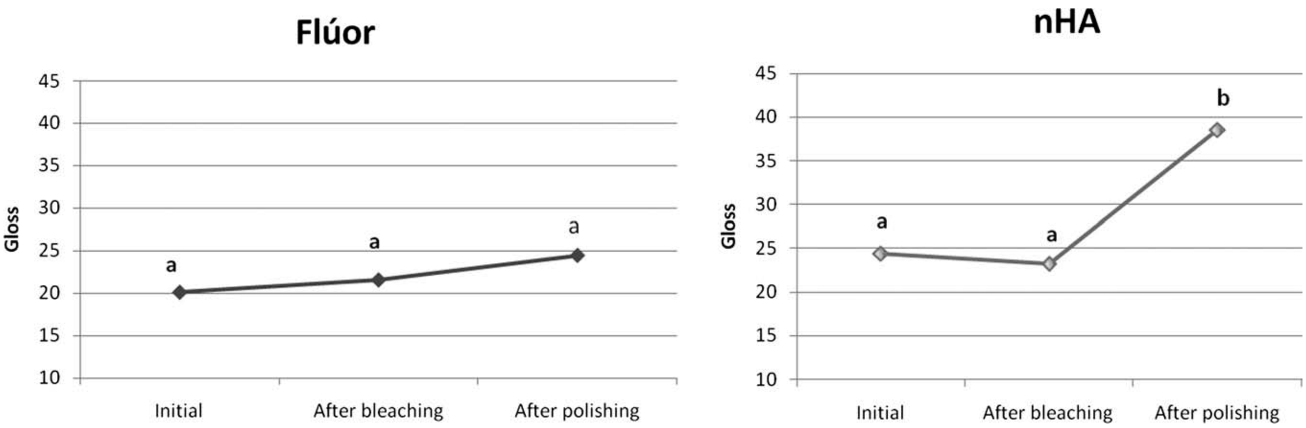

Fig. 6. Mean gloss values of Fluor and nHA groups before bleaching (initial), after bleaching and after polishing. ANOVA showed no significant difference ($P = 0.431$) for Group Fluor. ANOVA showed significant difference ($P = 0.001$) for Group nHA. Tukey's t-test is represented by the lowercases in the graphic.

that the sides and the base of the grooves became more irregular than the pretreatment grooves. The small irregularities on the surface can be attributed to normal enamel topography. Even PSD demonstrated that the contribution of all morphological wavelengths after bleaching procedure were similar to the initial PSD. In fact, the overlapping of PSD can also be used to validate the AFM measurements (Fang et al., 1997). Even though changes in enamel surface had already been found in AFM images after bleaching treatment (Hegedüs et al., 1999), this difference may be due to the time application, different concentration or different pH of bleaching agent.

As a matter of fact, surface roughness can have many effects on device properties depending on its spatial wavelength. This includes atomic level roughness which can affect gate oxide reliability, all the way up to longer wavelength features which lead to "haze." In the literature, the roughness measured by AFM is often expressed by simple statistical parameters, such as average roughness (Ra), root-mean square (RMS) roughness, or peak-to-valley height roughness (Z range), etc. In these cases, 250.000 pieces of information (512×512 pixels per image) are merely represented by a single number. Two images with exactly the same RMS values for example may have different surface morphology. This is because statistical measures such as RMS roughness are only sensitive to vertical, not horizontal structures. These statistical parameters are simple and reliable, but they only give information along the vertical direction and hence cannot fully characterize the surface. Therefore, using parameters which ignore spatial wavelength to describe roughness may present some problems. This will lead to difficulties in correlating roughness with device characteristics. Instead of using simple statistical parameters, AFM images can be represented by PSD over different spatial frequency regions. In this case, the PSD is advantageous because it allows comparison of the roughness data taken over various spatial frequency regions. Such methodology also offers a convenient representation of the direct space periodicity and amplitude of the roughness. In this study, the PSD results allow evaluating the contribution of each morphological wavelength, evidencing a decrease in all morphological wavelengths in the visible spectra range after polishing with dental tooth paste containing Nano<mHAP> agent. This result suggests that features with wavelength in the visible spectra range (Morphological wavelength) interact with visible light and thus are responsible for the optical properties of the surface. Unfortunately, a standard method to calculate power spectral density is not available (Fang et al., 1997). Hence, spectral analysis gives a more general and precise description than the standard roughness parameters alone (El Feninat et al., 2001).

Because of the extensive knowledge regarding the effect of fluoride on the remineralization process of dental tissues, the use of fluoride therapies concomitant with bleaching procedures could be helpful to saliva in remineralizing damaged dental tissues during and after bleaching (Leandro et al., 2008; Wiegand et al., 2007). According to the results found in this study, polishing bleached enamel surfaces with 2% neutral sodium fluoride did not significantly change Ra, RMS, and *Z* range mean values. The benefit effects of 2% neutral sodium fluoride after tooth bleaching on the decrease in surface roughness have been shown in the literature (Martin et al., 2010). However in this study is possible to notice that even PSD data showed a slight decrease in morphological wavelengths in the visible spectra range (especially close to 400–500 nm) after polishing with 2% neutral sodium fluoride, which probably might not be able to affect the interaction with visible light spectra.

Jeng et al. (2008) found that the roughness of the fluoride-treated enamel increased as a result of the growth of aggregates. However, following removal of the loosely bound deposits, the surface roughness was restored to a value close to that of the original native surface. Possibly in this research, the sonicated bath was able to remove the loosely deposits, showing no roughness difference after polishing with 2% neutral sodium fluoride.

After polishing with a dental tooth paste containing nano-sized hydroxyapatite, the only roughness parameter that evidenced statistical significant difference was *Z* range that really decreased after polishing. Despite Ra and RMS have not shown statistical significant difference, these roughness parameters evidently decreased too. These result might be due to the nano-hydroxyapatite ability of been deposited in the cavities and defects of enamel surface (Lv et al., 2007), enhancing smoothness. As the particle of nano-hydoxyapatite is fairly small sized, it can enter into the enamel surface continuously and fill the vacant position of enamel crystal. Although it is very dense, partial penetration of certain ions and molecules through the enamel structure is possible because it contains small and intercrystalline spaces, rod sheaths, enamel cracks, and other defects (Tanaka et al., 2010; Yamagishi et al., 2005). Roveri et al. (2009) noticed a thick and homogeneous apatitic layer covering the surface of the demineralized enamel after treatment with hydroxyapatite nanocrystals. The PSD obtained after polishing with tooth paste containing nano-sized hydroxyapatite shows a significant decrease in the contribution of all morphological wavelengths especially in the visible spectra range (380–750 nm), which leads to a smooth surface.

The morphological spatial frequency in the range of visible light spectrum (380–750 nm) is especially important in this study because it generates an interaction with the visible light. Accurate estimation of the location, form, and color of objects is challenging because the light arriving at the eyes depends on the spectra and spatial layout of the light sources on object properties. Focusing on the object surface, it can be absorptive, reflective or the surface can scatter the visible light. The visible light will be mainly scattered in surfaces with high PSD in the morphological wavelengths in the range of visible light spectrum (380–750 nm). Analyzing the spatial frequency in the range between 0.0013 nm^{-1} and 0.0025 nm^{-1}, which represents the same range of the visible light spectra (380–750 nm), we can observe a considerable decrease of the PSD after polishing in nHA group. This result promotes more visible light reflectance in specular component, which leads to a glossy surface. This is in agreement with gloss results which showed no gloss increase after bleaching treatment nor after 2% neutral sodium fluoride polishing. The only procedure that led to a gloss increase was polishing with dental toothpaste containing Nano<mHAP> agent. This result suggests that features with wavelength in the visible spectra range interact with visible light and thus are responsible for the optical properties of the surface, as gloss (Pedreira de Freitas et al., 2010). Surface texture controls the degree of scattering or reflection of the light striking on the natural tooth.

To the best of our knowledge, the PSD analysis of native, bleached or polished enamel surface presented in this study and its relation to gloss alteration, are not reported elsewhere in the literature. In our study we used AFM, which is a suitable microscopic method to analyze biological objects under natural conditions (Hegedüs et al., 1999). This condition is very important if we suppose that the organic phase of enamel plays an important role in the morphological changes of enamel after bleaching, in such case AFM can provide additional knowledge about the surface morphology of bleached enamel.

We can conclude that changes on surface morphological wavelengths, on the same order of magnitude of the visible light wavelengths, are responsible for the visual changes, like changes in gloss; seeing that gloss is an optical property, which is based on the interaction of light with physical characteristics of a surface.

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