The Effect of Remineralizing Toothpastes on Enamel Surface Roughness after Hybrid Laser Bleaching (An *In vitro* Study)

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ABSTARCT

Background: one of the complications of power bleaching is surface roughness of enamel which increases the possibility of post bleaching teeth discoloration. The aim of the present study is to evaluate the effect of toothpaste containing nano hydroxyapatite, NovaMin and kin sense fluoride on surface roughness of human tooth enamel after laser bleaching with 35% hydrogen peroxide bleaching gel.

Materials and Methods: Twenty human enamel incisors were cleaned and their labial surface polished up to #1200, then categorized into four equal groups; first group kept without bleaching as a control group, while the remaining three experimental groups were bleached with 35% hydrogen peroxide, and each group treated with a restore paste containing one of the following: nano hydroxyapatite, NovaMin, and Kin fluoride. Bleaching was done with laser hybrid system (DMC Whitening Lase II, Sao Paulo, Brazil). Enamel roughness values assessed by an atomic force microscopy (AA3000, Bosten, USA) before and after treatment with restore tooth paste.

Results: Paired t-test used to compare the mean roughness values before and after treatment with each restore paste. One-way analysis of variance and Duncan post hoc tests used to determine the differences between mean roughness values of the groups. A p-value of 0.05 or less considered a significant. The results showed a highly significant statistical differences of remineralization of all types of restore tooth pastes.

Conclusions: Nano hydroxyapatite past exhibiting a higher ability to reduce the surface roughness after laser bleaching than other tested pastes.

Keywords: Laser bleaching, remineralization, surface roughness, enamel. (J Bagh Coll Dentistry 2015; 27(4):1-7).

INTRODUCTION

Tooth whitening is a highly desirable esthetic treatment, as the tooth color is one of the most important factors related to the patients' satisfaction with their appearance [(1)]. Dental bleaching treatments are mainly based on the action of hydrogen peroxide, which is able to penetrate the tooth structure and release free radicals, oxidizing the chromophores molecules [(2)]. Such molecules are mainly organic, although inorganic molecules can also be affected by these reactions. Nevertheless, the free radical reaction is not specific and it may also alter the organic component of enamel [(3)]. Since the organic content contributes to the integrity of enamel, different adverse effects on both mineral and organic parts of bleached enamel have been observed [(4)].

Alterations in enamel surface morphology[(5-7)], chemical composition[(8-9)] and microhardness values[(2,10)], after previous reporting of bleaching. Furthermore, some changes in bleached enamel were also described as slight erosive effects promoted by the bleaching agent[(11)]. Nevertheless, some authors claim that the erosive pattern on the surface of bleached enamel only occurs when bleaching gels with low pH are used[(12,13)].

Attempts to minimize the adverse effects of bleaching treatments by increasing enamel remineralization have been conducted, however, the results are contradictory[(14)].

The association between the bleached enamel surface alterations and the subsequent susceptibility to erosive lesions resulting from the contact of bleached enamel with demineralizing solutions has been reported. The application of carbamide peroxide gel rendered enamel more susceptible to demineralization[(15)]. In other studies, at-home bleaching technique did not increase the susceptibility of enamel to erosion[(16)]. However, the effect of at-office bleaching agent (35% HP) on the enamel susceptibility to erosion that has not been properly discussed.

Considering the possibility that bleaching gels with high concentration of HP could increase the susceptibility of enamel to erosion, the addition of remineralizing ions into bleaching gels would be beneficial for preventing a further enamel demineralization [(17)].

Therefore, the objective of *in vitro* study was to evaluate the effect remineralizing tooth pastes gel on the bleached enamel with 35% hydrogen peroxide.

MATERIALS AND METHODS

Preparation of the Samples

Twenty extracted non-damaged and intact human incisors were stored in a 0.1% thymol solution and refrigerated at 4˚C, until required.

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Enamel samples prepared from the labial surface of the tooth using a diamond disc with straight-type micro motor handpiece (NSK, Tokyo, Japan) under coolant (Fig. 1).

Labial surfaces were polished to create a smooth and flat enamel surface with ascending-grit water proof silicon carbide papers starting from #400 up to #1,200 under running water. The specimens were immersed in deionized water and placed in an ultrasonic bath for 10min (Ultrasonic Cleaner, Odontobras, Ribeirao Preto, Brazil) for the removal of all waste, and then stored in thymol solution at 0.1% for rehydration.

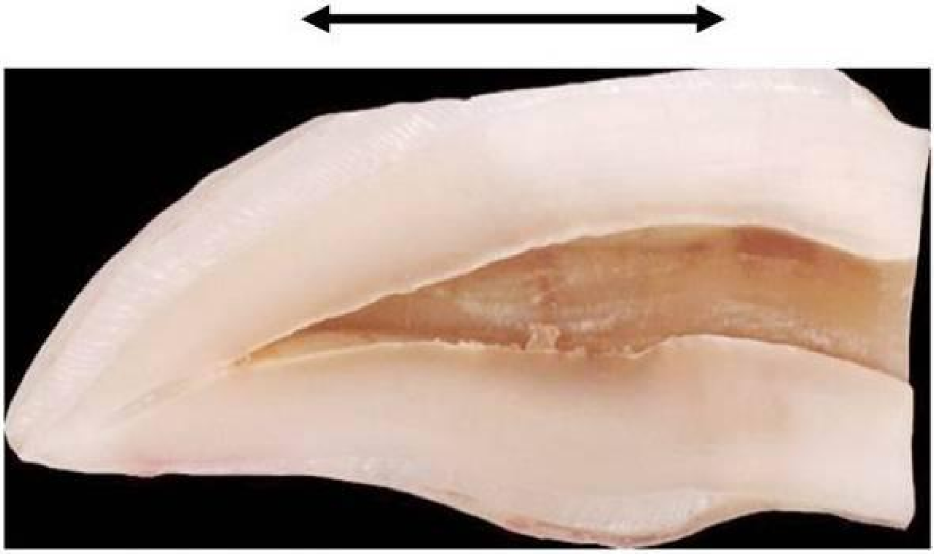

Fig. 1. Crosscut Surface of the Prepared Specimen.

Bleaching Procedures

The specimens were randomly assigned into four groups each comprising 5 enamel samples. One of the group was kept non-bleached as control, while the other three experimental groups were bleached with 35% hydrogen peroxide, before treated with nano hydroxyapatite (nHAP), NovaMin and kin fluoride toothpastes. After polishing procedure, the polished specimens covered with a masking tape of 5 mm-diameter to ensure measuring the same area of R_a value for polished, bleached and treated specimens with restore toothpastes (Fig. 2).

The bleaching procedure was performed using Whitening Lase II, DMC (810nm wavelength diode laser mix with LED). According to the manufacturer, the specimens rinsed with deionized water to remove the bleaching agent (20 sec).

Surface Roughness

After completion of bleaching process with 35% at office laser bleaching, the surface roughness R_a value was calculated for all bleached specimens. Such evaluation conducted by non-contact method using AFM (AA3000, Angstrom Advanced Inc. USA) through application of restore pastes according to the sample size to all bleached enamel sample by small brush with gentle brushing motion for 5 min, then washed and dried and R_a value calculated again for all treated enamel specimens for all groups. The differences in average (R_a) values between bleached and reminerlized enamel surface for each specimen were recorded and analyzed.

Statistical Analysis

Data analyzed with SPSS program version 21, 2013. Paired t-test used to compare the mean R_a before and after treatment with each restore paste. One-way analysis of variance and Duncan post hoc test used to determine differences in mean roughness values among the groups. The level of statistical significance was a p-value ≤ 0.05.

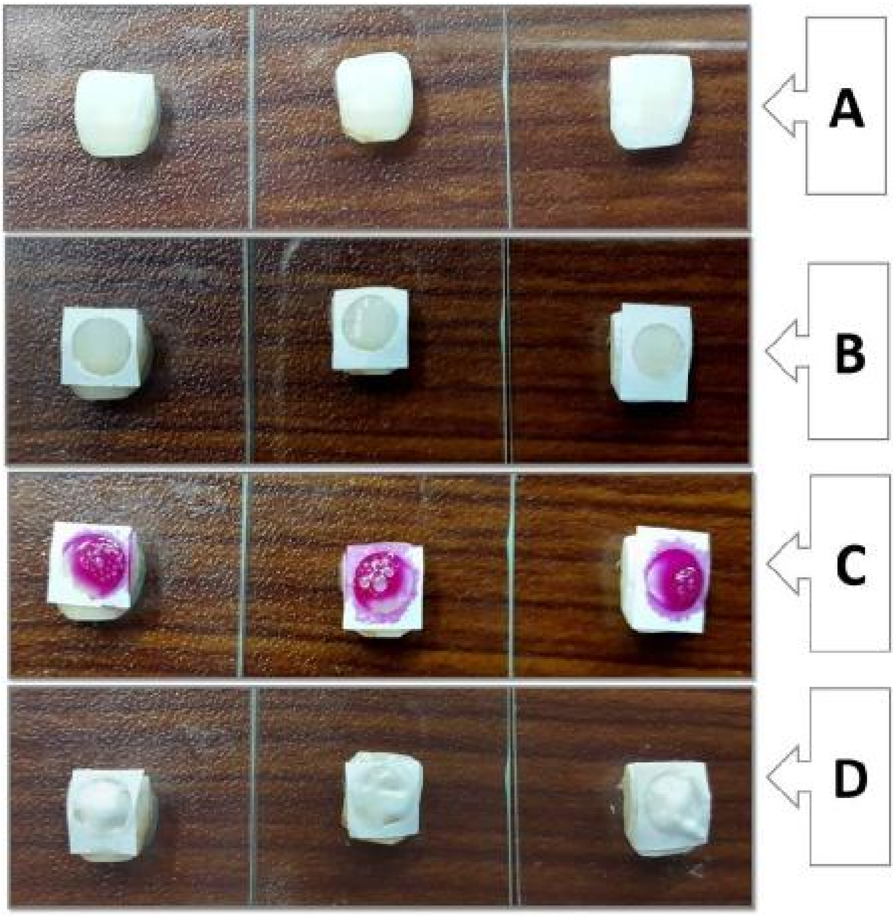

Fig. 2. A: Specimens of Polished Enamel up to 1200#. B: Specimen with Masking Tape with a 5mm-Diameter Hole. C: Specimen Bleached with 35%H_2O_2 Laser Bleaching (DMC). D:Specimen Treated with Restore Tooth Pastes.

RESULTS

Table1 and Figure 3 shows the mean, standard deviation and standard error of all groups after bleaching and treatment with restore tooth pastes.

A paired t-test used to assess whether there were any differences between pre & post treatment for each of the experimental groups. The results of roughness value were statistically analyzed as shown in table 2.

Table 1: Study Groups by Mean Roughness Value before and after Treatment with Restore Paste.

Study Groups	N	Mean R_a value	S.D.	S.E.
nHAP (A)*	5	37.340	0.427	0.191
nHAP (B)*	5	16.420	0.389	0.174
NOV (A)	5	37.300	0.529	0.236
NOV (B)	5	27.800	0.380	0.170
KIN fluoride (A)	5	37.120	0.729	0.326
KIN fluoride (B)	5	31.840	0.792	0.354
Control	5	18		

(A)* Before Treatment, (B)* After Treatment

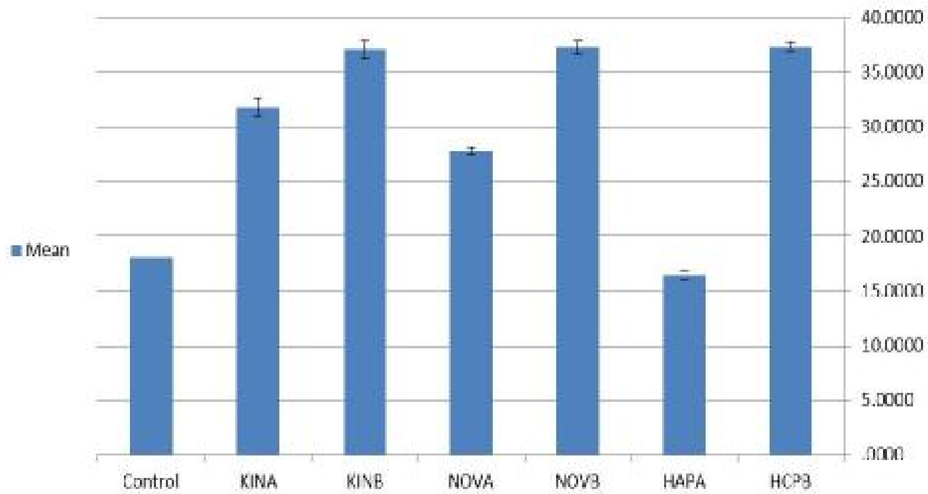

Fig. 3. Study Groups by Mean (R_a) before and after Treatment with Restore Paste

Table 2: Summary Statistics Comparing Mean Roughness Value before and after Treatment with Restore Paste

Study Groups	t-test	d.f.	Sig. (2 tailed)*
nHAP (pre)- nHAP (post)	284.685	4	0.000
NOV (pre)- NOV (post)	35.161	4	0.000
KIN (pre)- KIN (post)	7.833	4	0.001

***Based on Paired t-test**

Table 3: Differences between the Groups Using One-Way Analysis of Variance and Post Hoc Duncan Test.

	Sum of Squares	d.f.	Mean Square	F-test	Sig.
Between groups	2460.030	6	410.005		
Within groups	7.680	28	0.274	1494.810	0.000
Total	2467.710	34			

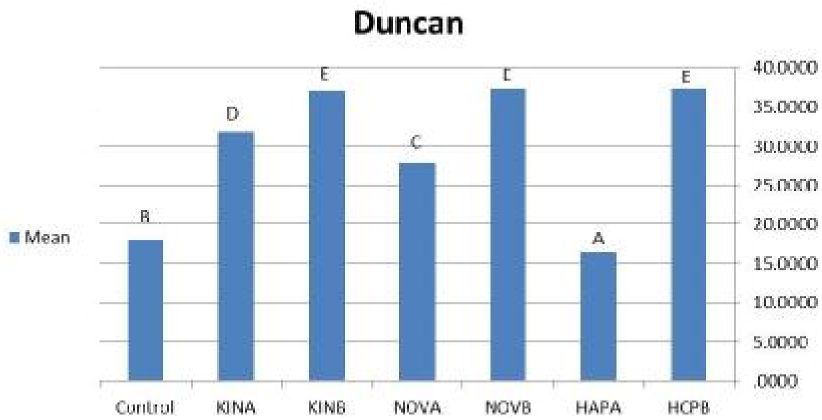

Fig. 4. Different Letters Indicated Significant Changes in Surface Roughness Value.

AFM Results

The Atomic Force Microscopy investigates the surface topography for all treated groups with various mean peak-to-valley height as show in Figures (5-9).

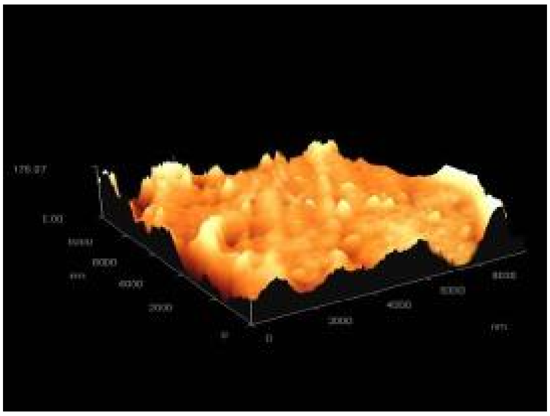

Fig. 5. 3D Surface Topography/ Control R_a Value = 18 nm.

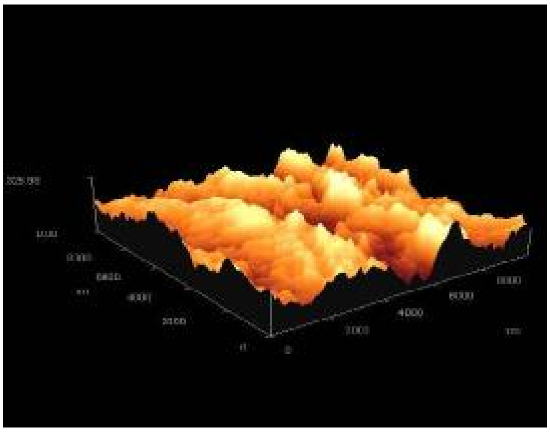

Fig. 6. 3D Surface Topography/ Post Bleaching with 35% H_2O_2, R_a Value = 37.2 nm

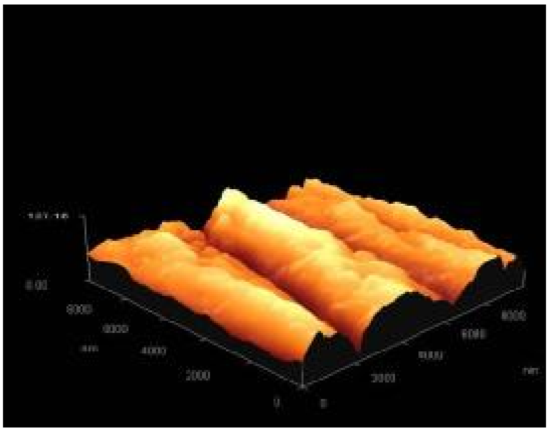

Fig. 7. 3D Surface Topography/Post Treatment with nHAP R_a value = 16.4 nm

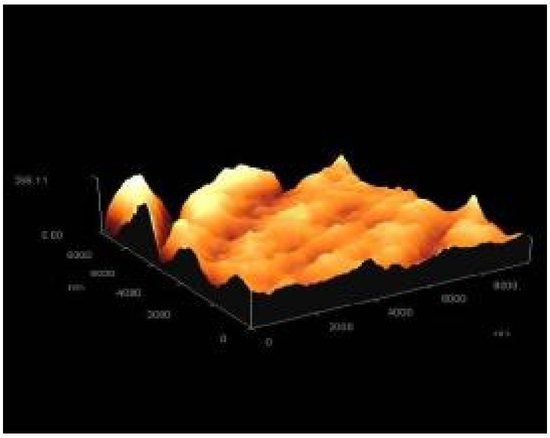

Fig. 8. 3D Surface Topography/Post Treatment with NOV. R_a value = 28.2 nm.

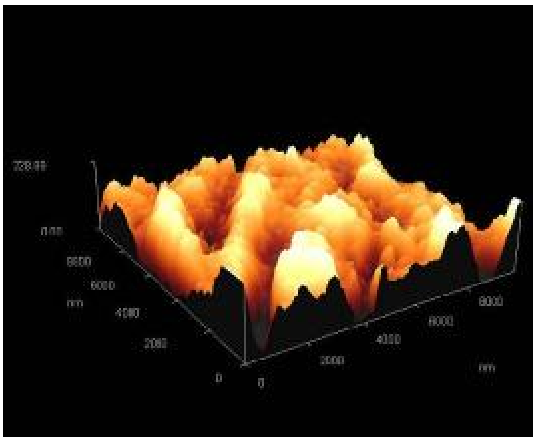

Fig. 9. 3D Surface Topography/Post Treatment with Kin sensi. R_a value = 32.6 nm.

DISCUSSION

Bleaching has become a popular procedure with seeking improvements in the perceived appearance of their teeth[18]. The effects of conventional bleaching using high concentrations of hydrogen peroxide on enamel's surface widely discussed in the literature, still presents conflicting results [19].

Some studies have claimed that there are no significant effects of bleaching agents on human enamel[20]. However, other studies have obtained contradictory results, with treated enamel showing morphological changes suggesting that bleaching is an erosive process[21,22]. Bleaching directly affects the organic (protein) content of the tooth, but this leads to changes in the mineral phase, resulting in the observed morphological changes to the tooth surface [4].

It may be possible to reverse this damage by the development of mineralizing agents to treat the affected tooth surface which are capable of bonding chemically to hard dental tissues such as oxides of calcium, sodium, phosphorous and silica in ratios that imparts bioactivity [23].

In the present study AFM/SPM used for determination of enamel surface texture as non-contact method, it is useful to study the surface texture at different magnification powers. Also, it provides a 3D images and roughness value for specimens.

The mean (R_a) values obtained after bleaching was (37.2 nm) approximately for all specimens. This can be explained by the fact that the enamel surface structure is composed of hydroxyapatite crystals and each crystal of Ca10 (PO4)6(OH)$_2$ is surrounded by a layer of tightly bound water. The presence of this hydration shell makes the crystal electrically charged and can therefore attract ions. Free radicals emitted from hydrogen peroxide during bleaching are able to play a part in demineralization and cause Ca+2 loss from enamel crystal, and this matches with the findings of Lewinstein *et al*[24] who concludes that peroxide could cause demineralization in enamel at low and high concentrations. Another study concludes that higher concentrations of hydrogen peroxide caused more Ca^{+2} losses than lower concentrations[18], this is also in accordance to the current study.

Atomic Force Microscopy reveals that exposing bleached enamel surface to different remineralizing restore pastes showed reduced surface roughness with significant differences between all groups.

Hydroxyapatite is the main component of enamel that gives the tooth a bright white appearance and eliminates the diffused reflection of light by filling up the fine pores of the tooth surface. Accordingly, remineralization of the teeth can be expected and its effect will be increased when the particle size of hydroxyapatite can be reduced to less than that of the micron-size in existing toothpaste preparations.

Hydroxyapatite materials 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[19]. Indeed, the interaction of Nanoparticles with dentine and enamel is more effective, due to the increased surface to volume ratio[25].

The results illustrate that the 3D Surface Topography/post treatment with nHAP R_a value (16.4 nm) is even less than the R_a value in the control group which is (18nm), this might be due to the nano-hydroxyapatite ability of being deposited in the cavities and defects of enamel surface[26] enhance 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[27,28]. Roveri *et al.*[29] notices a thick and homogeneous apatitic layer covers the surface of the demineralized enamel after treatment with hydroxyapatite nanocrystals.

The conclusion of the present study is that nano-hydroxyapatite had exhibit a higher ability to reduce the surface roughness after laser bleaching than other tested pastes. It is recommended to use it following laser bleaching to reduce the possible alteration on enamel surface.

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