

The Effect of Newly Introduced Bleaching Agent Listerine versus the Conventional Carbamide Peroxide on the Ultrastructure and Microhardness of Tooth Enamel

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Abstract

Background: Tooth bleaching is one of the most conservative and cost-effective dental treatments to enhance a person's smile. **Aim:** The aim of this study was to evaluate the effect of the newly introduced prebrush bleaching agent Listerine versus carbamide peroxide on the ultrastructure and micro-hardness of the enamel. **Materials and Methods:** Thirty non-carious sound human premolars extracted for orthodontic reasons. Teeth samples were divided equally into three groups. Group I served as control group where specimens were immersed in artificial saliva, group II treated with 22% carbamide peroxide for 5 sessions per day 15 minutes for 60 days and group III treated with Listerine pre-brush rinse for 5 sessions per day 15 minutes for 60 days, in between the sessions teeth were kept in artificial saliva. At the end of the experiment, all teeth were hemi-sectioned longitudinal in mesio-distal direction to form 60 specimens. The lingual halves of all the groups were used for measuring enamel micro-hardness, while buccal halves were examined by scanning electron microscope (SEM) to detect any ultrastructural changes of enamel of the different groups. **Results:** SEM results showed morphological alterations on the enamel surface of group II and group III compared with control group I as appeared surface pitting, porosity, irregularity, roughness, craters like depression and micro-cracks. Results of enamel surface micro-hardness showed no significant differences were found among the bleaching groups (II-III) ($p=0.371$). However, all bleaching agents produced a significant increase in the mean value of micro-hardness of enamel compared to the untreated control group (I) as there are highly significant between group I and other groups as group II ($p=0.003$) and group III ($p=0.026$). **Conclusion** bleaching abuse had a negative influence on the morphology of enamel and its micro-hardness. The home dental bleaching should be done with caution.

Key words: Tooth discoloration, tooth whitening, enamel roughness, artificial saliva.

Introduction

The natural color of teeth within the permanent dentition varies between individuals and represents a balance between the

pale yellow of dentine and translucency of the overlying enamel⁽¹⁾. Tooth discoloration or staining can be broadly classified as extrinsic or intrinsic. Extrinsic staining occurs when external chromogens are de-

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posited on the surface of the tooth or within the surface pellicle and can be direct or indirect. Intrinsic staining is a permanent tooth discoloration arising from a change to the structural composition of the tooth⁽²⁾. Bleaching treatment is considered to be the most conservative procedure for treating discolored and stained teeth when compared to laminate and crown restorations. Moreover, bleaching can be used to reduce the color of dark teeth before preparation and placement of aesthetic indirect restorations. Therefore, depending on color reduction, the tooth preparation can be more conservative and preserve more sound dental tissue^(3,4). Dental bleaching is a cosmetic dental procedure in which natural teeth are whitened with a bleaching agent such as peroxide. Bleaching agents are provided for in office or at home therapies. In-office bleaching requires less patient's cooperation, but longer sessions are necessary in the dental office, which increases the treatment costs. Otherwise, at home bleaching is less expensive, but patient's collaboration is essential to obtain successful treatment results⁽⁵⁾. Carbamide peroxide is used in many bleaching products; it breaks down into hydrogen peroxide (H_2O_2) (10% of carbamide peroxide yield approximately 3.5% of H_2O_2) and urea⁽⁶⁾. H_2O_2 decomposed into H_2O and O ; the latter is free radical associated with high permeability and diffusibility and permeates into enamel subsurface through the prismatic region and reacts with the organic matrix, pigmented molecules and removes it so it increases the surface irregularity^(7,8). Bleaching agents cause a decrease in enamel knoop micro-hardness, but values were recovered after treatment showing the importance of saliva in recovering mineral content, surface roughness was altered during or after treatment depending

on Hydrogen peroxide (HP) concentration with amorphous calcium phosphate (ACP) which has beneficial effects on surface roughness restricted to lower (HP) concentrations in association with remineralization effect of saliva⁽⁹⁾. Enamel surface changes after carbamide peroxide bleaching alternation include increase porosity, pitting, erosion and demineralization of enamel prisms periphery⁽¹⁰⁾. *Listerine*: Listerine is a brand of antiseptic mouth wash and it is one of the most popular mouth washes sold in the United States. The product is marketed under the slogan "kills germs that cause bad breath" it was named after Joseph Lister. Listerine's whitening pre-brush rinse is a product to help whiten teeth prior to brushing and this will help killing germs and will add a foaming agent to get in between teeth⁽¹¹⁾.

Materials and Methods

Sample preparation

Thirty non carious sound premolars extracted for orthodontic reasons were collected from the outpatient clinic of oral surgery department, Faculty of Oral and Dental Medicine, Suez Canal University from persons (15-20) years of age. Teeth were cleaned of gross debris and stored in artificial saliva until the time of their use. In this study, artificial saliva was used for simulation of the conditions given by natural human saliva. The teeth were divided equally into three groups as follows: *Group I*: Untreated samples (control group), consisted of 10 extracted teeth soaked in artificial saliva for 60 days. *Group II*: consisted of 10 extracted teeth. They were subjected to 5 sessions/day 15 minutes each of 22% carbamide peroxide for 60 days. In between the sessions, teeth were kept in artificial saliva. *Group III*: consisted of 10 extracted teeth. They were subjected to 5

sessions/ 15 minutes each of Listerine prebrush rinse for 60 days. Similarly in between the sessions teeth were kept in artificial saliva. At the end of the experiment, all the teeth of the different groups were hemi sectioned longitudinally in mesio-

distal direction with a low speed air and water cooled diamond disc. The lingual halves of all the groups were used for measuring enamel micro-hardness, while the buccal halves were examined by the scanning electron microscope (SEM) to detect any surface ultra-structural changes of enamel of different groups.

Bleaching Materials

The carbamide peroxide: was synthesized using urea (All Chemistry) and H_2O_2 130 ml (All Chemistry) in equal proportions. Using a mortar and pestle, crush 20 gm urea into a powder and then 35 ml of a 30% H_2O_2 was added the resulting mixture should have a dough consistency. Stir in 1.5 gm of powdered gelatine. The mixture was divided into two portions, covered with plastic wrap and kept at rest for 24h and 48h respectively, in the dark, resulting in the samples CP24 and CP48. After this period, both portions were vacuum filtered and the solids were stored in desiccators for 24 hours⁽¹¹⁾. To prepare 22% carbamide peroxide solution, 22 gm of solids of carbamide peroxide were mixed with 100 ml distilled water. *The listerine:* (Johnson and Johnson health care products. MCNEIL PPC. Inc. 2007. USA.)

Preparation of specimens for scanning electron microscopic examination

Buccal halves were prepared for SEM (JSM-5600; JEOL USA, Inc., Peabody, MA, USA) examination as follows: mounting on the metal stub by their cut surfaces using double sided adhesive tape, then enamel surfaces were coated under vacuum with

gold by S150A SPUTTER COATER Edwards. The chamber of coating admit argon gas through a dosing valve, then a glow discharge is ignited between the specimens table (anode) and the sputtering target (cathode) when high voltage is applied. The positively charged argon gas ions accelerated to the cathode as a result of this

process knock metal atoms free. The released ions and numerous gas molecules in the process chamber collide frequently, and the metal atoms are thus widely scattered. A diffuse cloud of metal atoms forms, from which atoms settle more or less uniformly onto the surrounding surfaces, including the enamel surfaces of the sections. This process forms a homogeneous thin metal film. Coated halves were then examined by the scanning electron microscope for the detection of any possible changes in the enamel of the experimental groups when compared to the controls. SEM examination was done in the electron microscopic department of National Research Center, Cairo, Egypt.

Micro-hardness measurement

Assessment of micro-hardness was done for enamel of the lingual halves of the different groups of teeth. Micro-hardness testing was measured by the Vickers Hardness tester (Shimadzu Micro-hardness tester HMV-2 Series, China) in the department of Solid State Physics at the National Research Center, Cairo, Egypt. The Vickers indenter is a square based diamond pyramid that creates a clear measurable indentation in the field as diagonals with two arms approximately equal in length. The hardness number can be converted into units of pascals, but should not be confused with a pressure, which also has units of pascals. The hardness number is determined by the load over the surface area of

the indentation and not the area normal to the force, and is therefore not a pressure⁽¹²⁾. A load of 100 grams was found suitable for this test for a loading period of 15 seconds and a loading speed of 0.017 mm/ second. The Vickers hardness number was calculated using the following formula: $VHN (kg/mm^2) = 185.4 \times P/d^2$, VHN = micro-hardness for Vickers, P = testing load in grams, d = length of the diagonal line across the indent in microns. The hardness number is not really a true property of the material and is an empirical value that should be seen in conjunction with the experimental methods and hardness scale used. When doing the hardness tests the distance between indentations must be more than 2.5 indentation diameters apart to avoid interaction between the work-hardened regions. The Vickers Pyramid Number (HV) is determined by the ratio F/A where F is the force applied to the diamond in kilograms-force and A is the surface area of the resulting indentation in square millimeters.
$$HV = \frac{F}{A} \approx \frac{1.8544F}{d^2}$$

Where A can be determined by the formula $A = \frac{d^2}{2 \sin(\frac{136}{2})}$ This can be approximated

by evaluating the \sin term to give $A = \frac{d^2}{1.8544}$

Where d is the average length of the diagonal left by the indenter. The corresponding units of HV are then kilograms-force per square millimeter (Kgf/mm^2). The hardness number can be also determined from special tables provided with the tester or digitally. Teeth were embedded in a thermoplastic resin as their outer surfaces (enamel surfaces) were being exposed outside the acrylic resin; the mounting of the samples in acrylic resin blocks was to facilitate placement and testing of the samples on micro-hardness tester. The load was 490 mN, the loading time was 15

sec, the distance between indentations was 1mm and number of Indentation made was 5 penetrations. Indentation were made in which the Vickers diamond penetrate perpendicular to the outer surface of the enamel. Micro-hardness of the enamel of lingual halve of teeth of different groups was determined digitally and the mean value of the readings was calculated (assuming that net change in hardness is directly correlated to mineral content). Readings of enamel micro-hardness were taken from several areas of the cut enamel surface⁽¹³⁾.

Statistical analysis

Collected data of the quantitative micro-hardness results were tabulated and subjected to statistical analyses by computer using the statistical package for Social Sciences (SPSS version 17) for windows. Descriptive statistics were used to calculate the mean and standard deviation of each group. One-way analysis of variance technique (ANOVA) was used in order to clarify if there was significant difference between the different groups. The p values < 0.05 were considered statistically significant.

Results

All groups were examined by: i) SEM Examination, ii) Enamel surface micro-hardness (M.H).

1- SEM Examination

The SE micrograph of group (I) showed revealed no specific structural defects. Low magnifications revealed intact, smooth enamel surface with fine scratches. The structural arrangement was characteristic of the normal enamel surface with no morphological irregularities (where alternating bands of rod ends and incremental lines of Retzius were seen).

Occasionally micro-pores were recorded Figure (1). Although the surface is not completely smooth, uniformity of the aprismatic surface layer can be observed. At higher magnification numerous bands of small depressions representing enamel prism core, margins and inter-prismatic substance alternating with parallel lines representing periky-mata (external manifestation of incremental lines of Retzius on enamel surface) were recorded. Enamel

surface alterations of group II and group III compared with control group I as appeared surface pitting, porosity, irregularity, roughness, craters like depression, micro-cracks, deep and very deep holes like lesions, exposure of enamel prisms with dissolution of their cores and partial removal of the aprismatic surface layer of enamel in certain areas resulting in indiscriminate erosions (Figures 2-5).

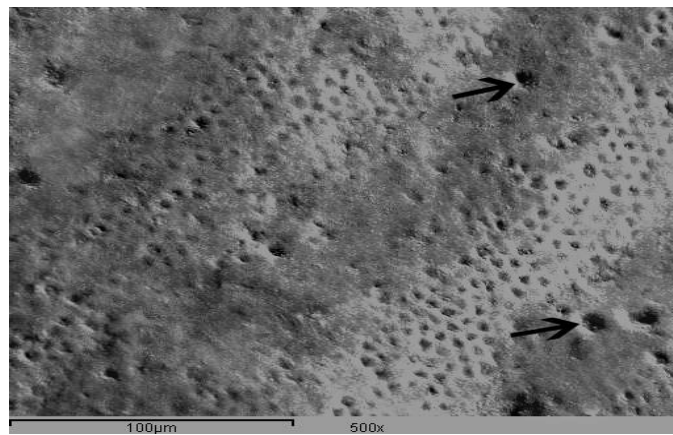


Figure 1: Scanning electron micrograph of enamel (control group I) showing the normal prism core, margins, inter-prismatic substance and occasional micro-pores (arrows) alternating with incremental lines of Retzius (*Orig. mag. 500*).

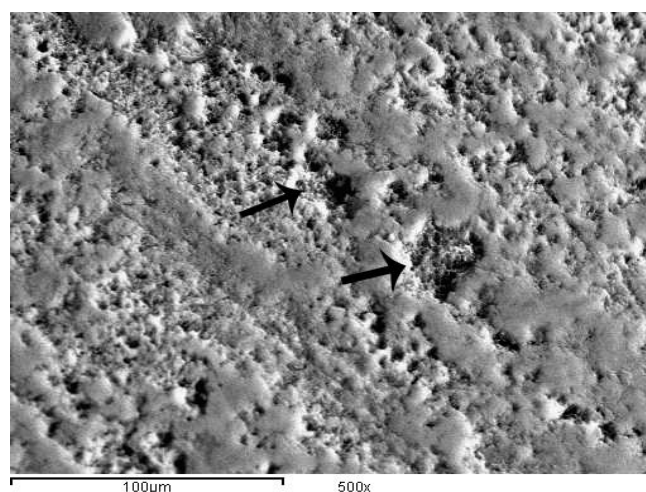


Figure 2: Scanning electron micrograph of enamel (group II) showing indiscriminate erosions (arrows) of enamel surface and surface roughness (*Orig. mag. 500*).

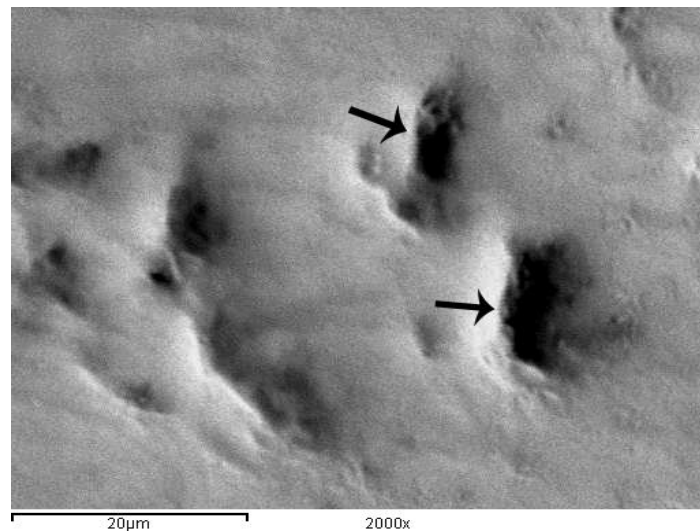


Figure 3: Scanning electron micrograph of enamel (group II) showing very deep holes in the enamel surface (arrows) (orig. mag. 2000).

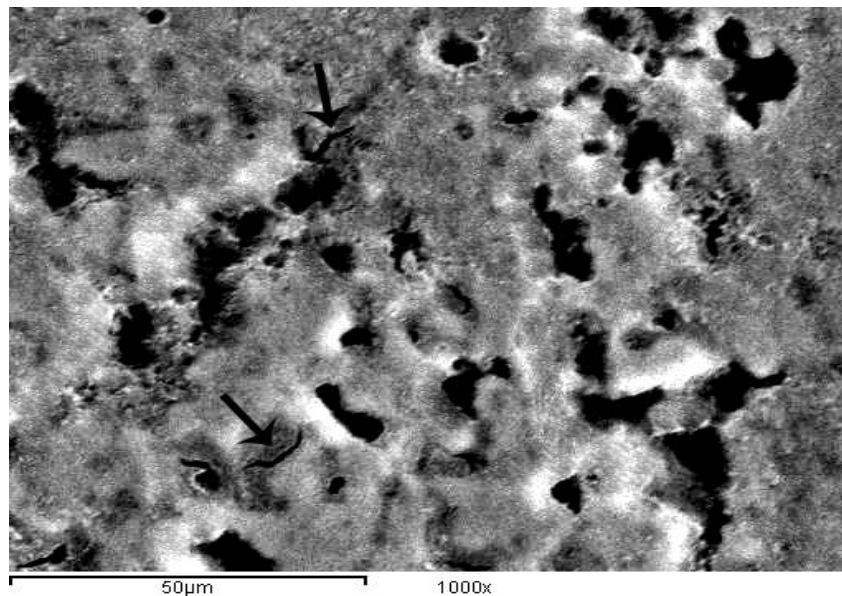


Figure 4: Scanning electron micrograph of enamel (group III) showing pronounced alterations and severe destruction of the surface with micro-cracks (arrows) and crater-like depressions in different depths (orig. mag. 1000).

2. *The enamel surface micro-hardness:*
After the bleaching procedure, data of the micro-hardness results were tabulated and

subjected to statistical analyses. Descriptive statistics and mixed designed One-Way ANOVA test was used to assess the statistical significance between more than

two study groups. Independent t-test was used to compare between two groups mean ($p < 0.05$). Vickers hardness numbers of same groups (II) ($p = 0.003$) and group

(III) ($p = 0.026$) were significantly lower than those of control group I with no difference among them (Table 1).

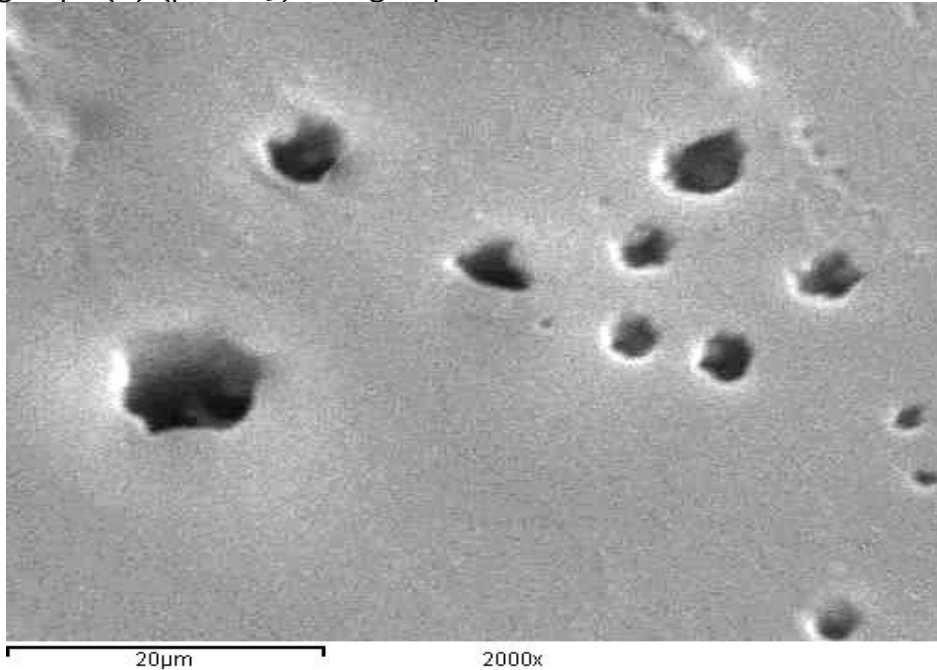


Figure 5: Scanning electron micrograph of enamel (group III) showing pores of various sizes and depths and dissolution of prismatic core (orig. mag. 2000).

It was concluded that 22% carbamide peroxide and Listerine had a potential harmful effect on enamel surface but Listerine more destructive as compared to 22% carbamide peroxide so caution should be warranted during and after its whitening procedures. Micro-hardness measurements showed decrease in the enamel sur-

face micro-hardness with bleaching enamel by 22% carbamide peroxide and Listerine.

Discussion

Today bleaching is an easy, conservative method to improve the esthetic appearance compared with other methods such as veneering or crown

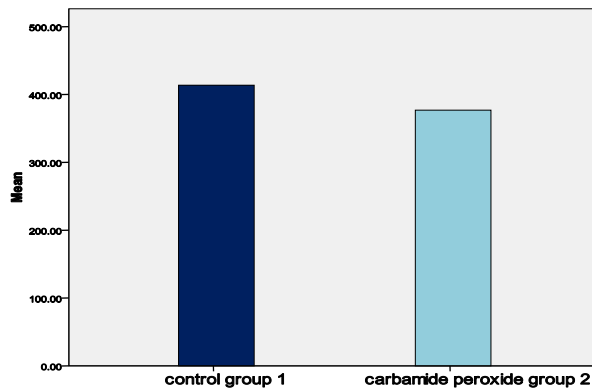


Figure 6: Mean Surface Micro-hardness between unbleached group I and bleached teeth with carbamide peroxide group II

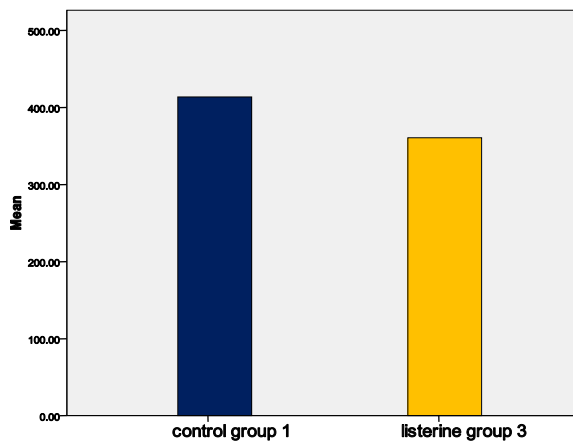


Figure 7: Mean Surface Micro-hardness between unbleached control group I and teeth bleached with prebrush bleaching agent Listerine group III ((p=0.371))

ing⁽¹⁵⁾. Most of the recent innovations in oral care products have been directed towards cosmetic marketing claims⁽¹⁶⁾. Although generally positive results have been reported concerning the whitening ability of these agents, concerns still remain as to their side effects on dental tissues⁽¹⁷⁾. In the present study, permanent, decay-free, adult erupted teeth extracted for orthodontic reasons were used; this may explain the occasional fissures on the enamel surface, aspects which were not influenced by the exposure to bleaching agents, since they were observed on the control samples as well. The results of the present

study showed that following bleaching, the enamel surface showed morphological alterations ranging from mild, moderate to severe, which is in accordance with the studies previously conducted⁽¹⁸⁾. In spite of the fact that all the two bleaching agents (22% carbamide peroxide and Listerine) used in this study contained the same active ingredient, i.e. H₂O₂, yet the surface morphology varied. This may be attributed to the presence of other ingredients in the agents, which are commercial secrets⁽¹⁹⁾. The dental bleaching using carbamide peroxide for long periods

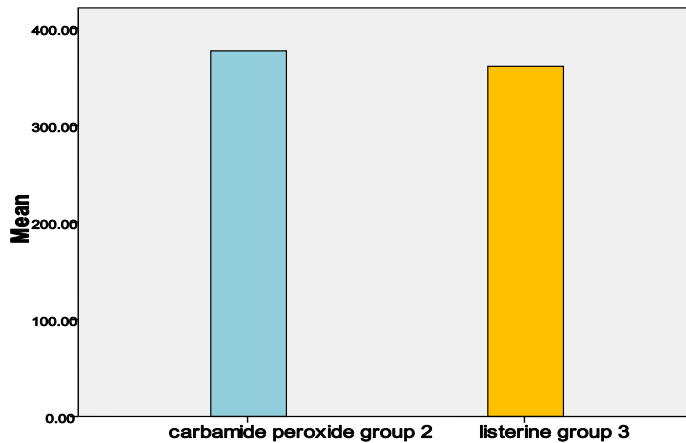


Figure 8: Mean Surface Micro-hardness between teeth bleached with carbamide peroxide group II and teeth bleached with prebrush bleaching agent Listerine group III.

Table 1. Multiple Comparisons

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% CI	
					Lower Bound	Upper Bound
Control	CP	36.83*	15.66	0.033	3.4372	70.2294
	Listerine	52.85*	15.66	0.004	19.45	86.24
CP	Control	-36.83*	15.66	0.033	-70.22	-3.43
	Listerine	16.016	15.66	0.323	-17.37	49.41
Listerine	Control	-52.85*	15.66	0.004	-86.24	-19.45
	CP	-16.016	15.66	0.323	-49.41	17.37

CP= carbamide peroxide

destroys different layers of enamel and produces loss of minerals. It has been reported that changes may occur not only on the enamel, but also in dentin and cementum after bleaching⁽²⁰⁾. Bleaching agents with H₂O₂ are believed to lighten the discolored tooth structure through decomposition of peroxides to give unstable free radicals which is Oxygen ions which are unstable, have short lifespan and react with other free substances or substrates presenting weak reactions. This is possible because of its high electro-negativity, which promotes a powerful reaction characterized by ions seeking molecular stabil-

ity. This process probably occurs due to the redox mechanism or a simple reduction promoted by the oxygen ion that reacts with the molecules that stain the teeth, becoming more simple, whiter or eliminated⁽²¹⁾. These free radicals breakdown the large pigmented molecules in enamel into smaller, less pigmented molecules through either oxidation or reduction reactions⁽²²⁾, while some studies suggested that such oxidation reactions cause alterations on the enamel structure^(23,24), other studies assumed that, bleaching agents have no adverse effects on the tooth structure⁽²⁵⁾.

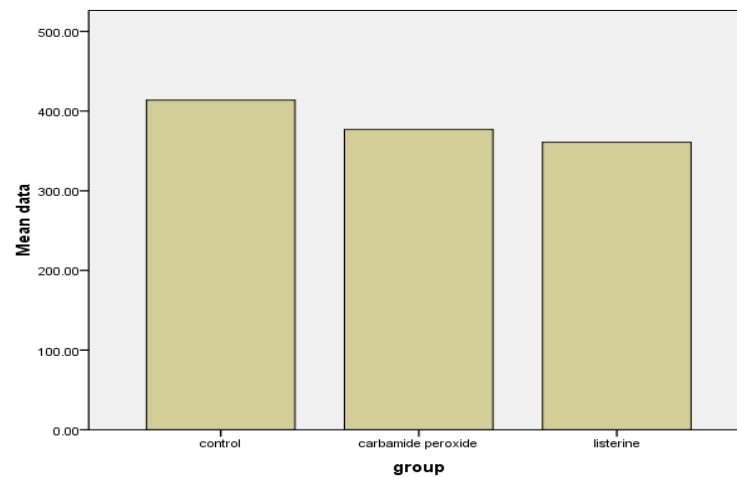


Figure 9: Mean Surface Micro-hardness between different groups

Still, this issue is debatable. Tooth whitening with Carbamide peroxide (CP) (group II) is one of most popular dental procedures. CP dissociates into H_2O_2 and urea when in contact with saliva at oral temperatures. Peroxide can diffuse through enamel and dentin due to its low molecular weight. H_2O_2 degrades into oxygen and water; urea degrades into ammonia and carbon dioxide. A general concern is expressed regarding possible weakening of the tooth structure⁽²⁶⁾. Other studies showed that some carbamide peroxide formulations caused calcium dissolution from enamel⁽²⁷⁾. Urea is a by-product of such bleaching agents and has been shown to be able to remove enamel proteins and related mineral elements⁽²⁸⁾, with the potential to penetrate through enamel and affect the prismatic and interprismatic structures contributing to the permeability increase and micro-structural changes⁽²⁹⁾. H_2O_2 is converted to perhydroxyl anion (HO_2^-) and free radicals, which destroy or oxidize the double bonds in the conjugated chain of chromophore⁽³⁰⁾. Both enamel and dentin are permeable by H_2O_2 and Carbamide peroxide. It was suggested that the peroxides could even

reach the pulp chamber⁽³¹⁾. Thus bleaching is not achieved solely by a surface effect⁽³²⁾, however the adverse effects of bleaching materials on the enamel surface are essential. A study with 20 and 10% carbamide peroxide and 25% H_2O_2 on canine and incisors was carried out and revealed no significant differences among the bleaching groups; however, all bleaching agents produced a significant increase in the mean surface roughness of enamel compared to the untreated control group⁽³³⁾. Significant surface alterations in enamel topography were detected in the present study SEM evaluation of specimens following Listerine application (group III). The 5 times daily application during the prolonged period of Listerine treatment (60 days) produced severe destruction of enamel surface integrity, irregularities, roughness, micro-cracks, pitting, stunting of enamel prisms, alto craters like depression with elevated peripheries, pores of different sizes and depths, extreme porosity, partial removal of the aprismatic surface layer of enamel in certain areas resulting in indiscriminate erosions and dissolution of prismatic core. SEM results of Varginha et al revealed re-

gional variation in tooth morphology surface with higher concentrations of H_2O_2 (up to 35%) that had tendency to promote an increase in density of pits and pores⁽¹⁷⁾. As a result of this increased surface roughness and irreversible changes it is possible that teeth may be more susceptible to extrinsic discoloration after bleaching or rinsing with whitening rinses. Another study evaluated the efficacy and safety of a whitening mouth rinse (2% H_2O_2) that was used daily during one week, the results showed very mild tooth color improvement but authors recommended to be careful with self-applied whitening products that contain peroxide since they have potential to produce oral irritation and tooth hypersensitivity⁽³⁴⁾. H_2O_2 gives an acidic solution with water thus rendering the pre-brush mouth rinse more acidic with low pH ranging from 3.0 to 3.8. Ponterfract et al measured enamel erosion by low pH mouth rinses, and reported that low pH mouth rinses should not be considered for long term or continuous use and never as pre-brush rinses⁽³⁵⁾. Although, Pretty et al monitored the erosive effect of several mouth washes including Listerine and found that it is the only one that caused any erosion compared to the negative control, but this was only significant after 14h of continuous use⁽³⁶⁾. As a result of bleaching regimens, the reduction in Knoop micro-hardness due to mineral loss can be naturally controlled by saliva and with re-mineralizing solution, such as artificial saliva which used as a storage medium to evaluate the re-mineralization potential of artificial saliva and whether it can restore micro-hardness of bleached enamel to normal or not⁽³⁷⁾ and fluorides⁽³⁸⁾ which reduces sensitivity by blocking dentin tubules and slowing fluid movement⁽³⁹⁾. So the presence of fluoride may enhance chemical reactions that lead

to the precipitation of calcium phosphate and decreased phosphate loss from enamel surface⁽⁴⁰⁾. The present results revealed that artificial saliva was not so powerful in restoring the micro-hardness of bleached enamel to normal and to counteract the demineralizing effect of the acidic bleaching agents used on enamel micro-hardness. The re-mineralization reaction is greatly enhanced by fluoride⁽⁴¹⁾.

Conclusions

According to the present results and within the limitations of this in vitro study, it could be concluded that: i) Home-use bleaching agents are capable of causing surface alterations. The peroxide-containing bleaching agents affect the enamel. Peroxide can affect not only the surface but also the inner structure of enamel. H_2O_2 can penetrate into enamel. Thus, inner oxidative effects are more likely to occur in the subsurface enamel where more organic material is present and oxidation is capable of altering the outer enamel surface. li) Whitening with 22% carbamide peroxide and Listerine pre-brush mouth rinse caused severe alterations on the enamel surface ultrastructure and decreased its micro-hardness, as they increase enamel surface irregularities, roughness, micro-cracks, pitting, stunting of enamel prisms, craters and pores of different sizes and depths. lii) Decrease in enamel surface micro-hardness may be responsible for easier penetration of cariogenic microorganisms and dissolution of the inorganic component of the dental tissues increasing the risk of dental caries.

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