Effect of bleaching teeth with hydrogen peroxide in the morphology, hydrophilicity, mechanical and tribological properties of enamel

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Abstract

The oral aesthetic medicine has contributed increasingly to correct aesthetic defects in order to respond to the standards of beauty imposed by society. A smile with white teeth is included in these standards. However, due to several factors, such as diet, age and certain vices, the natural color of teeth tends to change. The bleaching of teeth pretends to restore the original color of the teeth and consists in application of oxidizing agents, including hydrogen peroxide, on their surface. Although these products improve considerably the color of teeth, their effect on other properties of enamel is not yet fully understood.

This work aimed to study the impact of peroxide concentration used for tooth whitening in hydrophilicity, roughness, morphology, mechanical and tribological properties of human enamel.

With this purpose, human teeth were submitted to bleaching sessions with 6, 15 and 35% hydrogen peroxide, activated with UV radiation, to achieve maximum bleaching level B1. The enamel roughness and morphology was studied by AFM and SEM, the hardness using a microhardness tester, the hydrophilicity by the captive bubble method (as far as we know, never used to characterize teeth), and the tribological behavior using a nanotribometer.

The results show that, although the three solutions are able to improve the teeth color, they alter the properties of the enamel, inducing morphological changes, increase its roughness and wettability and decrease the hardness and wear resistance. It was found that the 15% hydrogen peroxide was the solution that less damaged enamel.

Keywords: Bleaching teeth, hydrogen peroxide, enamel, wear, wettability, mechanical properties

1. Introduction

The teeth are the hardest organ of human body and comprise four tissues: enamel, dentine, cementum and pulp [1]. Enamel includes 92-96% of inorganic substances (essentially hydroxyapatite) 1-2% of organic
materials as enamelin and amelogenin and 3-4% water [2].
The enamel microstructure is formed by prisms or rods with different orientations and closely packed [3]. These prisms consist of nanosized inorganic HAP crystals with different orientations inside and are covered by a nanometer thin layer of enamelin [3] [4]. The area between prisms is protein-rich and called inter-rod enamel [4].

Teeth aesthetic is very important for individual’s self-confidence and the dark teeth constitute a great problem. The darkening of the teeth occurs due to extrinsic and/or intrinsic factors. The former appear as a result of accumulation of chromophore substances, for example from food, drinks, poor oral hygiene and tobacco, in a pellicle which consists in an organic film that covers the external surface of the teeth. In contrast, the intrinsic factors are related with the teeth structure/composition, specially of dentin. Those stains are associated to aging, antibiotics use and tobacco, and are the most difficult to remove. [5] [6] [7]

Tooth whitening or bleaching is commonly used to restore the natural teeth color or to go beyond the natural color. The bleaches are oxidizing agents, based generally in hydrogen peroxide (H₂O₂) that reacts with dental enamel eliminating the coloring substances. The bleaching teeth process isn’t totally understood but some authors believe that the hydroxyl radical (·OH) cleaves the double bonds existing in chromophores making these molecules reflect less light, which produces a whitening effect [8] [9]. The peroxide radicals generated from H₂O₂ in enamel surface penetrate enamel, reacting with the pigments, and improve significantly the teeth’s color [10]. However, several studies have shown that bleaches lead to changes in the enamel surface and also enamel properties [11]. Most of these changes are probably due to low pH and oxidative effect of bleaching products [12]. These changes lead to a decrease in microhardness, alterations in morphology surface, variation in chemical composition and minor wear resistance [11].

The objective of the present study is to evaluate the effect of a bleaching agent (H₂O₂) in different concentrations, in properties like the hydrophilicity, roughness, morphology, mechanical and tribological behavior of human enamel.

2. Materials and Methods

2.1. Teeth preparation and initial color measurement

Five healthy human molars were extracted and stored in a thymol solution (0.1%) at 4°C in order to ensure aseptic conditions. After, the molars were cut longitudinally and then the cut surfaces were grinded and polished with 600, 800, 1000 and 2400 SiC sandpapers and clothes with 6, 3 and 1 µm diamond paste. Finally, the polished samples were cut in half in order to obtain 4 pieces with a polished surface area. The original color of all parts was determined by using a color spectrophotometer (SpectroShade™
Two measurements were done in each part. All the plates were then stained during 48 hours in 10 mL of black tea solution (2 g in 50 mL) at room temperature. After that, the samples were rinsed and kept immersed in DD water at 4ºC.

2.2. Bleaching and color control

After, the stained samples were treated in a solution of H₂O₂ as bleaching agent. The samples were submitted to 4-11 bleaching treatments, depending on the peroxide concentration, to obtain a maximum whiteness (shade B1). Each treatment consisted in the immersion of the dental sample in 10 mL of H₂O₂ solution during 10 minutes. In order to activate the peroxide, the immersed samples were irradiated during 30 s with ultraviolet radiation (λ=350 nm) every 2.5 minutes (total time of irradiation 4x30s). After each treatment, the sample color was measured. The differences in color during bleaching were calculated using the following equation [13]:

\[ \Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \]  

where \( \Delta E \) is the Euclidean distance between 2 points in the 3-dimensional space of shade and corresponds to the difference in sensation of color alteration, the coordinate \( L \) represents the shade alterations in the black and white scale, \( a \) represents shade and saturation in the red-green axis, and \( b \) represents saturation in the blue-yellow axis [13]. \( \Delta L, \Delta a, \Delta b \) represent the difference between the coordinates \( L, a, \) and \( b \) at the beginning and at the end of the experiment.

2.3. Roughness and Morphology

The surface roughness (Ra) was determined from Atomic Force Microscopy (AFM) images (100 x100 μm²) obtained with a microscope Nanosurf Easyscan2 with silicon tips (PPP-NCLAuD-10) and a load of 20 nN. It was used the software WSxM 5.0 Develop 4.0 to analyze the images.

The surface and wear mechanisms were identified by scanning electron microscopy observation (Hitachi S2400 equipment). The samples were previously coated with gold.

2.4. Microhardness measurement

The samples hardness was measured using a microhardness tester (Struers Duramin) with an applied load of 1.96 N and a dull time of 30 s. Fifteen measurements were performed per condition.

2.5. Wettability

The water contact angle measurement was done by the captive method using a goniometer. Air bubbles (3–4 μL) were generated with a micro syringe with an end curved needle underneath the polished surface of the teeth. Images were acquired at set time intervals during 30 s using a video camera (JAI CV-A50) mounted on an optical microscope (Wild M3Z) and
connected to a frame grabber (Data Translation DT3155). The acquisition and analysis of the images were performed using the ADSA-P software (Axisymmetric Drop Shape Analysis Profile). Eight to twenty-four drops/bubbles were done for each sample.

2.6. Friction coefficient measurements

Reciprocating pin-on-plate tests were performed in a nanotribometer (CSM Instruments), where the plates were the flat dental samples and the pins were 3 mm diameter zirconia balls. The tribological operational conditions are given in Table 1.

Table 1. Wear test operational conditions.

<table>
<thead>
<tr>
<th>Applied load (mN)</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroke distance (mm)</td>
<td>1</td>
</tr>
<tr>
<td>Frequency (Hz)</td>
<td>2</td>
</tr>
<tr>
<td>Duration (min)</td>
<td>10</td>
</tr>
<tr>
<td>Max Lin. Speed (cm/s)</td>
<td>0.65</td>
</tr>
</tbody>
</table>

The tests were performed in artificial saliva at room temperature. The composition of saliva is given in table 2.

Table 2. Chemical composition of artificial saliva.

<table>
<thead>
<tr>
<th>Reagents (g/L)</th>
<th>Concentration (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>0.600</td>
</tr>
<tr>
<td>KCl</td>
<td>0.720</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.166</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.680</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>0.337</td>
</tr>
<tr>
<td>KSCN</td>
<td>0.060</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>1.500</td>
</tr>
</tbody>
</table>

For each group of samples, ten wear tests were performed, i.e. two tests in each part.

3. Results and discussion

3.1. Effect of bleaching with peroxide in color

The difference in sensation of color alteration, ΔE, during the bleaching treatment is given in Figure 1. The results show that the number of applications decreases with increasing of percentage of hydrogen peroxide. The number of applications ranges between 4 to 35% concentration and 11 to concentration of 6%.

The total application time depending on the amount of peroxide can be adjusted to a power function. The determination coefficient (R²) obtained range between 0.98 and 0.99 (Figure 2). The same trend was previously reported by Sulieman et al [14].

According to Kwon et al. the mechanism that leads to changes in tooth color involves the diffusion of the H₂O₂ radicals in the tooth structure and the interaction of H₂O₂ radicals with chromophore molecules [15]. Since free radicals are low molecular weight, they can easily penetrate through the enamel inter-prismatic spaces following the Fick’s second law, i.e, with increases of peroxide concentration, more free radicals penetrate inside the tooth, accelerating the bleaching process. The results in Figure 2 confirm this theory, i.e., with higher concentrations more rapidly reaches its maximum whitening, B1. This result is also shown in other studies [16] [17].

The mechanisms by which UV radiation and peroxide bleach teeth can involve
breaking of bonds C–O, H–O and HO–OH because of by UV radiation energy (3.5 eV), the absorption of photons by peroxide leading to cleavage and formation of free radicals that it will interact with the chromophore and absorption of photons by the chromophore molecules that increases the energy of its C=O bonds, C=C and C=C–C=C making them more reactive to the peroxide [15].

When the saturation point in bleaching is reached, in which all the chromophore molecules are broken down and the maximum degree of whitening is achieved, the peroxide starts to interact only with organic molecules from the tooth structure causing damage to the tooth.

**3.2. Effect of bleaching with peroxide in morphology**

Morphological enamel changes can affect not only shine, but also bacterial adhesion and tribological behavior.

Analysis of the AFM images presented in figure 3 allowed concluding that the initial surface roughness of the teeth was 36 ± 23 nm (table 3).

After bleaching, the roughness of all surfaces increased. The roughness of the enamel surface ranged from 58 ± 17 nm for the 15% to 162 ± 15 nm for 35%. The peroxide solution 6% increased roughness to 107 ± 21 nm.

SEM images of untreated samples show the smear layer, i.e. small particles on the surface (Figure 4). For 6% and 15% is possible to see that the surfaces have more bites, leading to increased roughness. The samples treated with the peroxide solution 35% present randomly distributed defects across the entire surface, being observed the formation of localized erosion zones. These results are similar to those reported by Miranda et al [18].

The changes in the roughness of the enamel surface and morphology after exposure to hydrogen peroxide have been reported by other authors for solutions to 6% [19], 15% [20], and 35% [18].

Morphological changes may be associated with enamel demineralization due to the low pH of the bleaching solutions. It is known that enamel, when exposed to solutions

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**Figure 1.** Difference in sensation of color alteration, ΔE, during bleaching application.

**Figure 2.** Relationship between treatment time and hydrogen peroxide percentage to achieve a ΔE 8 and 10.
with lower pH 5.5, begins to dissolve, i.e. occurs demineralization, and the pH of three peroxide solutions are below this (pH = 1.16 in 35%; pH = 2.48 in 15% e pH = 3.49 in 6%). This occurs because H⁺ reacts with OH⁻ of hydroxyapatite equilibrium to form H₂O. With the decrease of OH⁻ concentration the equilibrium is displaced, so more Ca²⁺ and PO₄³⁻ will dissolve to achieve a new state of equilibrium.

### 3.3. Effect of bleaching with peroxide in mechanical properties of enamel

Enamel microhardness is directly related to the mineral content and structure of enamel, its degree of heterogeneity, the preparation of the tooth and the type of tooth [68,85].

The results of microhardness measurements are in Table 3. Before treatment the microhardness of enamel was 359 ± 19 VH. This value is in agreement with the literature, although there are some variations [21].

After treatment, occurs a significantly decrease of hardness to ranging the average value between 207 ± 20 to 35% and 235 ± 15 to 15%.

Other studies reported the decrease of microhardness of the enamel after exposition to peroxide in three concentrations tested: 35%, 15% and 6%. Other studies have shown no difference in hardness, and this may be due to the exposure time, pH of peroxide and application mode.

### Table 3. Values of microhardness, friction coefficient, roughness inside and outside of wear track and their wear volume before and after of different treatments.

<table>
<thead>
<tr>
<th>Tooth treatment</th>
<th>Ra in surface (nm)</th>
<th>Ra in wear tracks (nm)</th>
<th>Microhardness</th>
<th>Friction Coefficient</th>
<th>Volume tracks (μm² x 10⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without treatment</td>
<td>36±23</td>
<td>39±21</td>
<td>359±19</td>
<td>0.36±0.05</td>
<td>0.005±0.0004</td>
</tr>
<tr>
<td>6% H₂O₂</td>
<td>107±21</td>
<td>139±20</td>
<td>225±20</td>
<td>0.45±0.07</td>
<td>4.4±0.7</td>
</tr>
<tr>
<td>15% H₂O₂</td>
<td>58±17</td>
<td>94±25</td>
<td>235±15</td>
<td>0.40±0.07</td>
<td>2.5±0.3</td>
</tr>
<tr>
<td>35% H₂O₂</td>
<td>162±15</td>
<td>131±21</td>
<td>207±20</td>
<td>0.54±0.08</td>
<td>4.4±0.4</td>
</tr>
</tbody>
</table>

Figure 3.AFM Images of enamel surface before a) and after treatment with 6 b), 15 c) and 35% d) peroxide.

Figure 4.SEM Images of enamel surface before a) and after treatment with 6 b), 15 c) and 35% d) peroxide.
Loss of mechanical strength after bleaching may be attributed to demineralization, degradation of the organic matrix and the consequent increase of porosity and structural changes.

3.4 Effect of bleaching with peroxide in hydrophilicity of enamel

The study aimed to evaluate how changes in enamel surface due to the effect of bleaching treatments will change its hydrophobicity. Knowledge of the water contact angle is important because it can influence the absorption of water, future treatments of dental restoration, the tribological behavior and adhesion of bacteria and biomolecules.

![Figure 5. Contact angles without treatment (WT), after stained with tea and after bleaching treatment at different H₂O₂ concentrations.](image)

Before treatment, the average contact angle was 25° ± 3.6°. The initial contact angle is in agreement with the results obtained by Weizhong et al [22]. Other authors reported higher values of the order of 55.3° to 80° [23]. These differences are related to the surface preparation and the method of measurement used.

The results show that there is a slight reduction in contact angle after treatment with tea and bleaching. However, from a statistical point of view, there are no significant differences between the values obtained after immersion in tea and after the different treatments or between the different treatments.

Measurements after immersion in tea led to a decrease in angle to 24° ± 3.0°. After the bleaching treatment, the results vary between 23° ± 2.7° to 15% and 23° ± 3.0° to 6% and 35%. Up to date, as far as we know, no one has studied the effect of tooth whitening on enamel hydrophilicity.

The slight decrease of contact angle after exposure to black tea indicates a slight increase in surface hydrophilicity. This suggests that the hydrophilic groups of the chromophore adsorbed molecules shall interact with water.

Already slight decrease of contact angle after bleaching may be due to increased surface energy caused by the low pH of the peroxide solutions. As explained above, the enamel demineralization leads to the increase in surface roughness and a consequent increase in surface free energy which will lead to a decrease in contact angles.

The action of free peroxide radicals can also influence the surface free energy. It would be needed more research to better understand these results.

3.5 Effect of bleaching with peroxide in tribological properties of enamel

The objective of this analysis is to evaluate the tribological behavior of the enamel before and after exposure to peroxide. This is important because a high wear will result in the poor performance of the teeth during chewing.
In this work, in vitro tribological tests were carried out in a nanotribometer using a simple geometry (ball/plane) in lubricated medium (artificial saliva). The wear resistance of the enamel was evaluated using zirconia balls as counterbody (ZrO2) whose hardness is higher than the enamel (HV 1200).

The initial applied compressive contact stress obtained by application of Hertz equation for elastic contact was 158 MPa, which is within the masticatory pressure. In fact, in the literature the mastication force varies between 70 to 700N [2]. In addition, if it is considered an occlusal contact area of 2mm², the compressive stress varies between 35 and 350 MPa during mastication. In the occlusal contact the sliding distance is around 0.9 to 1.2 mm.

Also the tests were performed in lubricated medium in artificial saliva to mimetic the oral conditions.

The results obtained show that the application of the bleaching treatment leads to an increase of the average coefficient of friction and alterations over time (Table 3) (Figure 6).

The average coefficient of friction before treatment was 0.36 and after exposure to peroxide, increased to values comprised between 0.40 to 15% and 0.54 to 35%.

The average friction coefficient for the untreated samples is of the same order of magnitude as observed by other authors [24], about 0.4, though the experimental conditions are not exactly the same.

The increase in the average friction coefficient after treatment has also been previously reported by other authors [1]. This increase is consistent with the increase in the average roughness after treatment, leading to increase of the mechanical interlocking between the asperities of the sliding surfaces (mechanical term) in coefficient of friction.

Moreover, the slight reduction in contact angle after the whitening led to a more hydrophilic surface which corresponds to...
higher adhesion forces in coefficient of friction when in contact with the zirconia which is also hydrophilic.

Nevertheless, in all samples treated with 6% peroxide, there was an abrupt increase in the coefficient of friction at about 150 seconds. This behavior may be associated with a transition from moderate regime of wear, which is characterized by small wear particles to large particles in a more severe regime. In treatments with 15 and 35%, the friction coefficient remains high and relatively constant over time, being the highest observed for the 35% concentration.

The whitening leads to a decreased wear resistance of the enamel. From Table 1 analysis, it can be concluded that the wear volume was 67 times higher than the initial to 15% and about 80 fold to 6% and 35%. Mundra et al also reported an increase using bovine teeth and steel balls.

The increased of wear after treatment may be related to the decrease of hardness due to demineralization and loss of organic material during treatment.

The SEM observation revealed the existence of particles adhered to the surface. Some particles are small, with size of hydroxyapatite crystals, other has a lamellar form (Figure 7). The formation of "smear layer" after polishing is reported in various studies [18].

It is also visible gaps that should have been produced by subsurface fatigue phenomena (delamination) with consequent formation of lamellar particles. This phenomenon is associated with higher wear rates. For concentrations of 6 and 15%, the wear by fatigue is associated with a further increase of the roughness of the tracks, compared with what was observed outside of the tracks (Figure 7). To 35% increase in roughness was more evident outside the tracks.

Delamination also existed on the tooth surface after treatment leading to a large increase in roughness out of the wear track and also higher that inside of the tracks.

It should be noted that the results of this study shows that delamination may occur by chemical attack or tribological action.

Conclusions

The bleaching of the teeth with hydrogen peroxide causes:

a) An increase of surface roughness. The smallest increase in roughness was achieved with 15% of H₂O₂;

b) A decrease of hardness of the enamel. The differences between the hardness for the three concentrations are small, but it can be seen that, on average, the samples subjected to treatment with 15% have the lowest reduction;

c) A slight change in enamel surface hydrophilicity. There was a slight decrease in contact angles relative to the initial state of the enamel;

d) An increase in the friction coefficient and wear. The peroxide at 35% was the largest contributor to the increase of these parameters. The lower volume of wear was obtained for 15%.

For the studied concentrations, the bleaching solution at 15% is the one that
produces minor changes in enamel properties after bleaching.

References


