Effect of nanohydroxyapatite on bleached human enamel

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Abstract: Aim of the study: to investigate whether the application of nano-hydroxyapatite after bleaching differs from the routine used amorphous calcium phosphate (ACP) on surface roughness of human enamel. Introduction: Although bleaching is considered a non-destroying and safe method for teeth discoloration, many side effects including surface roughness and loss of mineral content. So every effort was done to remineralize tooth surface after bleaching, so the aim of the study was to investigate whether the application of nano-hydroxyapatite after bleaching differ from the routine used amorphous calcium phosphate (ACP) on surface roughness of human enamel.

Methodology: Teeth selection: A total of 63 freshly extracted non carious human permanent teeth were used for this study. Teeth were cleaned by using ultrasonic scaler (Cavitron Focused Spray slim LINE 1000 (30K), teeth were cleaned using a low speed hand piece (NSK, Japan), a polishing brush and a non-fluoride polishing paste (i-FAST, i-dental, UAB) under water coolant. The teeth were then stored in 0.5 thymol solution in an incubator (Kugel et al., 2007), with pH=7 until being used. Specimen grouping: A total of 63 teeth were included in this study. All specimens were subjected to bleaching application. Specimens were divided according to remineralizing agent application into three main groups according to remineralizing agent application. Group 1: (control) no remineralization; Group 2: GC mouse-er mineralizing agent and Group 3: Remin PUR-X remineralizing agent. Each group was further subdivided into three subgroups according to storage period into: immediate, 1 week and 4 weeks aging periods. Results: Concentration of phosphate was slightly greater than calcium and the maximum concentration for both calcium and phosphate was at 1 week group using Remin PUR-X remineralizing agent and period of aging did not have a significant effect on surface roughness and mineral content. Conclusion: It was concluded that Remin showed less surface roughness than ACP-CPP and Remin showed higher mineral content than ACP-CPP.

Keywords: ACP-CPP, Remin PUR-X, GC mouse.

1. Introduction:

The constant search and demand for an esthetic and harmonious smile as well as the concerns by professionals in solving dental chromatic alterations have been increased in the last decades. Bleaching systems had increased rapidly and showed wide interest by the dental practitioners to achieve esthetic result.

Although bleaching is considered a non-destroying and safe method for teeth discoloration (Roveri et al., 2009). There are concerns on the adverse effects of bleaching agents on enamel. One of the most known side effects for bleaching is surface roughness.

Such an increase in the enamel roughness might be due to the oxidation process of the enamel surface and the loss of the organic matrix which constitutes less than one percent of the enamel composition, due to the exposed high concentration of hydrogen peroxide bleaching agent, that might have caused dissolution of the superficial layer of the enamel surface and the low PH values (high acidity) of the bleaching agent because of the higher concentration of the hydrogen peroxide.

Also bleaching has adverse effects inducing tooth sensitivity caused by loss of minerals. This tooth sensitivity usually starts during bleaching and ceases 24–48 hours post procedure Sa Y et al., 2013. So every effort was done to remineralize tooth surface after bleaching to decrease mineral loss following bleaching treatment and this can be done through application of different remineralizing agent following bleaching procedure. One of the most known biomaterials that helped in remineralization on enamel surface is amorphous calcium phosphate (ACP). It is a biomaterial added to toothpastes and mouth rinse. It
precipitates rapidly on tooth surface and undergoes hydrolysis to form apatite crystals, filling the defects. As a result of this process, it is possible for dental tissue remineralization to occur (Tschoppe et al., 2009). Amorphous calcium phosphate (ACP) remineralizing agent on the tooth surface following bleaching procedure could stabilize the calcium and phosphate ions level, through maintaining a state of super saturation (Walsh LJ et al., 2006). ACP is an excellent delivery vehicle available in a slow release form of an amorphous form to localize calcium and phosphate ions at the enamel surface and has a synergetic effect in enamel remineralization (Kielbassa et al., 2009). It could buffer the free phosphate and calcium ions activities maintaining a supersaturation state by decreasing demineralization and promoting remineralization.

Nano-hydroxyapatite (nano-HAP) was introduced as remineralizing agent following bleaching procedure. It has a bioactive and biocompatibility factors which enable it to act as a template once it is applied to the enamel surface and filling the micro-pores by attracting a large amount of the phosphorus and calcium ions from the remineralization solution (Eva Klaric Sever et al., 2018). The rate and amount of nano-hydroxyapatite (nano-HAP) precipitation is increased at the surface along with the precipitation of calcium and phosphate ions and enhancing the remineralization effects (Eva Klaric Sever et al., 2018).

The use of 10% nano-hydroxyapatite (nano-HAP) appeared to be an optimal concentration for remineralization of enamel surface as the concentration of the nano-hydroxyapatite (nano-HAP) increased (Eva Klaric Sever et al., 2018).

2. Materials and methods:
Preparation of specimens:
A total of 63 freshly extracted non carious human permanent teeth were used for this study. Teeth were cleaned by using ultrasonic scaler (Cavitron Focused Spray slim LINE 1000(30K) (Haung S et al 2011). and were inspected using magnifying lens (Bausch and Lomb, Opt. Rochester, NY, USA) (Sidhu SK et al 2008) at 6X magnification to ensure that they are free from any defects, fractures or hypocalcification. After that, the teeth were cleaned using a low speed hand piece (NSK, Japan), a polishing brush and a non-fluoride polishing paste (i-FAST, i-dental, UAB) under water coolant. The teeth were then stored in 0.5 thymol solution in an incubator, with pH=7 until being used.

40% hydrogen peroxide bleaching and remineralizing agent application:
According to manufacturer’s instruction, an in-office chemically-activated bleaching gel composed of 40% hydrogen peroxide (Power, white smile, Discuss Dental, Germany, 13138) applied over the enamel surface of all specimens of (1-2) millimeter thickness and spread over the surface. The bleaching cycle was of fifteen minutes. The bleaching cycle was repeated three times so that the total bleaching time for all specimens was 45 minutes for each specimen, before application of remineralizing agent. Specimens were then divided according to remineralizing agent application into three main groups (21 each). Group one (control) represents bleached enamel surface with (no remineralization), group two represents bleached enamel surface coated with (ACP-CPP) MI Paste (GC-tooth mousse) (GC, Tokyo, Japan) (Kielbassa AM et al 2008) as remineralizing agent on the surface and group three represents bleached enamel surface coated with nano-HAP (Remin PUR-X) as remineralizing agent. Specimens of each group were then subdivided into three subgroups according to aging, in which subgroup one: immediate, subgroup two: 1 week and subgroup three: 4 weeks. Surface roughness and mineral content was then evaluated. For the immediate group, the specimens were stored in artificial saliva for only 24 hours after bleaching, while for the one week and the seven weeks group, the specimens were stored in artificial saliva at 37 degrees. The teeth were kept in an incubator (Shel lab, Sheldon Manufacturing, Inc. Cornelius, OR, USA) (Tschope P et al, 2013). The saliva was changed daily to prevent bacterial growth. It was adjusted at 37°C degrees according to storage period either for 1 week or 4 weeks, pH of the solution was checked using a digital pH meter (Hanna, Milano, Italy).

Surface roughness measurements:
Stylus profilometer
All specimens were tested for surface roughness after bleaching and after remineralizing agent application with the different storage periods using a contact stylus profilometer (DE Abreu DR et al., 2011) (Talyscan 150, England). The need for quantitative characterization of the surface topography treatment was done by maintaining the contact with the surface. Each specimen was traced mesio-distally with the predetermined reference area 3 times at approximately the midline of the sample in the middle third of the labial surface for a length of 1.5 mm. It used a probe to detect the surface, physically moving a probe along the surface in order to acquire the surface height. This is done mechanically with a feedback loop that monitors the force from the sample pushing up against the probe as it scans along the surface. A feedback system is used to keep the arm with a specific amount of torque on it, known as the ‘setpoint’. The changes in the standardized position of the arm holder can then be used to reconstruct the surface. Each specimen was traced mesio-distally 3
times at approxiametly the midline of the sample. Stylus profilometer involves physical movements in three definite points while maintaining contact with the surface where it is slower than non-contact techniques.

**Mineral content measurement:**

**Energy Dispersive X-ray (EDX) microanalysis:**
Quantitative analysis (determination of the concentrations of the elements present) entails measuring line intensities for each element in the sample and for the same elements in calibration standardized of known composition. By scanning the beam in a television-like raster and displaying the intensity of a selected X-ray line, element distribution images or 'maps' can be produced. Also, images produced by electrons collected from the sample reveal surface topography or mean atomic number differences according to the mode selected. The scanning electron microscope (SEM), which is closely related to the electron probe, is designed primarily for producing electron images, can also be used for element mapping, and even point analysis, if an X-ray spectrometer is added. This allowed producing characteristic X-ray lines, the bombarding electrons also give rise to a continuous X-ray spectrum (section 2.4), which limits the detachability of small peaks, owing to the presence of 'background'.

Materials’ specifications, Composition, Lot number and manufacture:

<table>
<thead>
<tr>
<th>Product</th>
<th>Ingredients</th>
<th>Lot number</th>
<th>Manufacture</th>
</tr>
</thead>
<tbody>
<tr>
<td>White smile-Power In-Office (whitening gel)</td>
<td>Water, 40% Hydrogen peroxide, hydroxyethyl acrylate/sodium arloylidimethylitaure copolymer, etidronic acid, potassium stannate, ammonium hydroxide</td>
<td>18234</td>
<td>Weinheimer Straße 6 69488 Birkenau Germany WHITE smile, GmbH</td>
</tr>
<tr>
<td>Remin paste X-PUR Nanohydroxyapatite (nano-HAP)</td>
<td>10% nano medical hydroxyapatite (mHAP), SLS free, 10% xylitol, non-medical ingredients: (macrogol 400, zeolite, polyvinlypyrrolidone, glycyrthetin acid, cetylpyridinium chloride, glycerin, xylitol, dimethysilyl silicic anhydride, castor oil, sodium lauroyl glutamate, carragenan, ethonol, carboxymethylcellulose sodium, titanium dioxide, flavor)</td>
<td>63635000380</td>
<td>Sangi Co., LTD Tokyo, 104-8440, Japan</td>
</tr>
<tr>
<td>GC tooth mouse MI paste</td>
<td>Casein Phosphopeptide Amorphous Calcium Phosphate, Pure Water, Glycerol, D-Sorbitol, CMC-Na, Propylene Glycol, Silicon Dioxide, Titanium Dioxide, Xylitol, Phosphoric Acid, Zinc Oxide, Sodium Saccharin, Ethyl P-Hydroxybenzoate, Propylyp-Hydroxybenzoate, Butylp-Hydroxybenzoate.</td>
<td>170210B</td>
<td>GC Corporation, Tokyo, Japan</td>
</tr>
</tbody>
</table>

**Statistical analysis:**
All results were collected, tabulated and statistically analyzed.

3. Results

**Analysis of surface roughness:**
Mean and Standard deviation of surface roughness values for the three studied groups after one week aging period, with the specimen of group I reading had a mean value of (3.66 ± 1.04), group II (GC mouse) had a (2.78 ± 1.02 ) mean value and the third group (Remin PUR-X) showed a (2.18 ± 1.03) mean value. Pair-wise Duncan post-hoc test showed a significant difference between tested groups at p=0.047. The control group recorded higher statistical non-significant surface roughness mean value than group II (GC mouse) at p value= 0.271. The mean value of the control group was statistically significantly higher than group III (Remin PUR-X) at p value=0.039. The mean value of group II (GC mouse) was statistically non significantly higher than group III (Remin PUR-X) at p value=0.537.

<table>
<thead>
<tr>
<th>Surface roughness</th>
<th>Control</th>
<th>GC mouse</th>
<th>Remin PUR-X</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min. – Max.</td>
<td>2.61 – 5.43</td>
<td>1.66 – 4.43</td>
<td>1.15 – 3.80</td>
<td>3.642*</td>
<td>0.047*</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>3.66 ± 1.04</td>
<td>2.78 ± 1.02</td>
<td>2.18 ± 1.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>3.33</td>
<td>2.44</td>
<td>1.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig. bet. groups</td>
<td>p₁=0.271, p₂=0.039 , p₃=0.537</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean and Standard deviation of surface roughness values for the GC mouse group after different aging periods. Pair-wise Duncan post-hoc test showed that showed a significant difference
between tested groups at p value=0.117. The immediate (control) group showed the highest statistical significant surface roughness mean value followed by the one week group while the four weeks group recorded the lowest mean value (p< 0.05), while it showed that the differences between (1 week versus 4 weeks) was statistically non-significant (p>0.05).

**Table (2): Comparison between the studied periods according to Surface roughness in GC mousse:**

<table>
<thead>
<tr>
<th>Surface roughness</th>
<th>Immediate</th>
<th>1 Week</th>
<th>4 Weeks</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC Min. – Max.</td>
<td>2.61 – 5.43</td>
<td>1.66 – 4.43</td>
<td>1.64 – 4.20</td>
<td>2.421</td>
<td>0.117</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>3.66 ± 1.04</td>
<td>2.78 ± 1.02</td>
<td>2.53 ± 0.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>3.33</td>
<td>2.44</td>
<td>2.03</td>
<td></td>
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</tr>
</tbody>
</table>

F: F for ANOVA test, Pair wise comparison between each 2 groups was done using Post Hoc Test (Tukey) p: p value for comparing between the studied groups

Mean and Standard deviation of surface roughness values for the for Remin PUR-X group after different aging periods. Pair-wise Duncan post-hoc test showed a significant difference between tested groups at p value=0.009*. The immediate group statistically significantly higher than 1 week group at p value=0.034. Surface roughness mean value of the immediate group was statistically significantly higher than 4 weeks group at p value=0.011. Surface roughness mean value of 1 week group was statistically non significantly higher than 4 weeks group at p value=0.845.

**Table (3): Comparison between the studied periods according to Surface roughness in Remin PUR-X:**

<table>
<thead>
<tr>
<th>Surface roughness</th>
<th>Immediate</th>
<th>1 Week</th>
<th>4 Weeks</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remin Min. – Max.</td>
<td>2.61 – 5.43</td>
<td>1.15 – 3.80</td>
<td>0.80 – 3.30</td>
<td>6.249*</td>
<td>0.009*</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>3.66 ± 1.04</td>
<td>2.18 ± 1.03</td>
<td>1.88 ± 0.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>3.33</td>
<td>1.83</td>
<td>1.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig. bet. groups</td>
<td>p&lt;0.034*, p&lt;0.011*, p=0.845</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

F: F for ANOVA test, Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey)

**Figure (1):** A drawn model predicted estimating the mean difference in surface roughness of GC tooth-mousse after different aging periods, with the specimen of immediate group reading had a mean value of (3.66 ± 1.04), 1 week group had a (2.78 ± 1.02) mean value and the fourth week group showed a (2.53 ± 0.97) mean value. The control group (immediate) showed the highest value. The fourth week group showed the lowest value, with no significant difference between group II and group III.

**Figure (2):** A drawn model predicted estimating the mean difference of the surface roughness Remin PUR-X after different aging periods. The control group (immediate) showed the highest value. The fourth week group showed the lowest value, with no significant difference between group II and group III.

**4. Discussion:**

The results of the present study showed that bleaching treatment significantly increased surface roughness due to decrease in enamel hardness without the use of remineralizing agent and artificial saliva. It
may be possible to reverse the damaging effect of bleaching by the use of remineralizing agents to treat the affected tooth surface as stated by Heshmat H et al., 2016. Enamel surface demineralization could be controlled by the action of saliva, artificial saliva, remineralizing solutions and fluoride.

In order to, overcome these side effects of bleaching. Enamel surface demineralization could be controlled by the action of saliva, artificial saliva, remineralizing solutions and fluoride.

Regarding surface roughness, the assessment of surface roughness in this study is important due to the fact that this property affects light reflection, color fading, appearance of cracks and aesthetics in addition to biofilm accumulation Mirzaie Met al., 2016. The results of the present study indicated that control group with no remineralizing agent applied over the surface showed the highest statistical significant surface roughness values compared to the other groups without remineralizing agent applied to bleached enamel surface.

Another study by Attia Met al., 2015 attributed to the process of remineralization on bleached enamel surface to improve enamel micro-roughness, where artificial saliva and CPP-ACP contain calcium and phosphate minerals. Accordingly, remineralization of the teeth can be expected and its effect will be increased when particle size of hydroxyapatite can be reduced to less than that of the micron-size in existing toothpaste preparations, where mean surface roughness of nano-hydroxyapatite recorded the lowest statistical significant surface roughness. According to ANOVA test results, the micro-roughness mean value of all groups, after bleaching showed a significant difference compared to before bleaching, which means that bleaching can increase micro-roughness of enamel regardless to procedure. Regarding the use of nano-hydroxyapatite, the result of the present study revealed that the nano-hydroxyapatite showed significant lower surface roughness than the two other groups with higher micro-hardness, showing that nano-hydroxyapatite had preserved the enamel surface from demineralization. These results was in accordance with the study made by Gomes et al., 2017 where nano-hydroxyapatite paste was found to promote the formation of a protective layer, reducing the possibility of enamel demineralization caused by hydrogen peroxide.

However, Attin et al., 2009 reported that because hydroxyapatite contained nano-metric particles, nano-hydroxyapatite was able to penetrate these erosive areas and fill it to avoid further demineralization and promote remineralization with the deposition of calcium phosphate hydroxide. The same results was obtained by Abreu et al., 2011 where it was determined that CPP-ACP addition could decrease enamel surface roughness, but could not improve enamel hardness (Vashisht et al., 2013 and Balakishnam et al., 2013), this was due to CPP-ACP is able to stabilize calcium phosphate 110 times better than liquid solution at neutral pH and base before undergoing spontaneous precipitation process as stated by Ozkan et al., 2013 where CPP-ACP remineralization process begins with the attachment and deposition of ACP in CPP-ACP at the surface and in the inter-prismatic gap of enamel.

In this study a contact method of measurement using a tracing device and night guard with a hole of (1x1 mm) diameter situated in the middle third of the labial surface of the bleached enamel surface. This method was used to standardize surface area of measurement for accurate readings before and after material application using contact profilometer and stylus for tracing the surface physically.

Regarding surface roughness measurements, using profilometer which measured small surface variation in vertical stylus displacement as a function of position. This allowed the surface profile to be studied at different magnification process Mathias et al., 2009. Also, contacting the surface is often an advantage where non-contact method can end-up measuring surface contaminant instead of surface itself.

Regarding aging, surface roughness and mineral content, it was shown that aging assumed better results when compared to immediate group. On the other hand, there was no difference between one and four weeks in surface roughness.

Using Pair- wise Duncan post-hoc test revealed highest statistical non-significant mineral content % mean value followed by 1 week group while immediate group recorded the lowest statistical significant mineral concentration % mean value (p> 0.05) & the difference between 1 week group and 4 weeks groups was non-significant (p> 0.05).

In other word, natural human saliva revealed better protective effect on human enamel structure than distilled water. The chemical reaction continued to reach maximum after one week and then non-significant increase after the four weeks. So, it was concluded that artificial saliva would provide data closer to reality and closer to the clinical situation.

Conclusion:

Under the limitations of the present in vitro study, the following conclusions were drawn:

- Remin showed less surface roughness than ACP-CPP
- Aging have a positive effect on surface roughness
- The period of aging did not have a significant effect on surface roughness
References:
17. Pages 343-348 | Received 12 Dec 2011, Accepted 26 Feb 2012.; 08 May 2012.

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