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THE INFLUENCE OF CHROMOPHORE PRESENCE IN AN EXPERIMENTAL BLEACHING GEL ON LASER ASSISTED TOOTH WHITENING EFFICIENCY

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ABSTRACT. The aim of this study was to assess the ability of chromophore to improve laser whitening procedures on bovine teeth. The teeth were previously stained with black tea. We evaluated the color change with the help of the Vita Easy Shade Spectrophotometer. Two batches of four teeth were prepared and immersed for 14 days in a solution of black tea. For the bleaching, an experimental gel was used. Batch I was kept in the gel without chromophore and batch II in the gel with chromophore. The teeth from both batches were kept for 30 sec in the gel and were afterwards irradiated with a diode laser (980nm). The statistical analysis showed significant differences between T1 (the measurement of the color after the staining process) and T2 (the measurement of color after the whitening process) for the first batch and T0 (the initial measurement of the color of the teeth) and T2 (the measurement of color after the whitening process) for the second batch.

Keywords: *dental bleaching, laser, bovine teeth, experimental gel*

INTRODUCTION

Due to recent increase of aesthetic expectations, the methods to achieve a brighter, whiter and beautiful smile have also seen a continuous development. Patients come more often to dental clinics in order to achieve

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the results that make them feel more comfortable and more beautiful. The color of their teeth is a very important aspect so they try to achieve lighter shades. Studying the enamel, the shade change could be the result of two processes: the demineralization and the direct absorption of the food dye.

After the teeth eruption, their initial color also changes because of the interaction with different types of food dyes, smoking, and various chemicals found in drugs, bacteria, as well as other types of pigments. In time, the pigments start to infiltrate the dental structures, changing their shade. The main purpose of the chemical treatments used to obtain a lighter shade is to release chromophore compounds into the dental structure. The most commonly used dental bleaching agents are hydrogen peroxide (H_2O_2), hydrogen peroxide urea ($CH_6N_2O_3$), sodium perborate ($NaBO_3$). The hydrogen peroxide urea decomposes and determines hydrogen peroxide.

Polyphenols representing the coloring substances may determine two types of reactions. One of them is the binding reaction that is done by chelating with calcium ions found in the structure of the hydroxyapatite in the enamel and in the dentine. The second possibility is of forming compounds due to the reaction of collagen found at the level of the dentine.

The two types of bounds are attacked by compounds called free radicals. These are the result of decomposed hydrogen peroxide. The chromophores are later transformed into small-molecule compounds. This change makes them easier to be removed from dental structures.

The decomposing of peroxide leads to intermediary products such as the free radicals ($H_2O_2 \rightarrow H\cdot + \cdot OOH$) or ($H_2O_2 \rightarrow HO\cdot + \cdot OH$), it can also generate perhydroxyl anions ($H_2O_2 \rightarrow H^+ + :OOH^-$) or combinations between free radicals and anions, also basic ($\cdot HOO + OH^- \rightarrow O_2^{-}\cdot + H_2O$) or acidic ($HOO\cdot \rightarrow O_2^- + H^+$) solution. Under the effects of peroxides, the oxidation takes place ($C{=}C + O\cdot \rightarrow C{-}C + H_2O \rightarrow OH{-}C{-}C{-}OH$) [1].

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The main reaction of the mechanism involved in the bleaching process is the redox reaction where the hydrogen peroxide performs a reduction of the organic pigments that are impregnated in the dentin and in the enamel, thus allowing its elimination. When in contact with the dental enamel, the hydrogen peroxide releases oxygen that is unstable, and that will form links with other substances that are free or unstable bounded to a substance, in order to obtain the stabilization. The reaction takes place because of the large electronegativity of the oxygen. The oxygen ions react with the molecules that stained the enamel. As a result they break them and generate single chains or smaller molecules, which are lighter and therefore are easier to eliminate [2].

Researches in the recent years were focused on improving the process of teeth whitening by combining the effects of whitening gels with lasers [3].

The activation of the bleaching agents can be done with light sources such as Er:YAG, Nd:YAG, diode, argon, Co_2 lasers, Led and halogen lamps. Colorless bleaching gels do not absorb significantly the light sources used for the activation and therefore a big amount of energy is reflected or transmitted. Using a colored bleaching agent provides the absoption of the light by the bleaching gel [4,5,6,7].

The diode laser is absorbed in precise chromophores found in the composition of beaching gels. Its absorption makes the reaction of bleaching to take place more rapidly, more effectively and more safe if we compare it with the conventional method [8].

The aim of this research was to assess and compare the efficiency of two formulas with and without chromophore for the same experimental gel during laser-assisted bleaching procedures on bovine teeth.

RESULTS AND DISCUSSION

1. Assessment of luminosity (L*) parameter indicated statistically significant differences between at least two stages for batch I ($p < 0.01$) and between at least two stages for batch II ($p < 0.001$).

As expected, for batch I, luminosity (L*) values were higher in T0 than in T1 or T2 and in T2 than in T1. All the differences were statistically significant. The same situation was observed for batch II: higher values in T0 than in T1 or T2, and higher values in T2 than in T1, all the differences being also statistically significant ($p < 0.001$).

There were no statistically significant differences between the two batches in any of the stages of this study ($p>0.05$).

2. For the red-green contrast (a*) values, statistically significant differences were observed between at least two stages for batch I ($p < 0.001$), and also for batch II ($p < 0.001$).

For batch I, the red-green contrast values (a*) were higher in T1 and T2 than in T0, the differences being statistically significant. Between T1 and T2, the differences were not statistically significant. For batch II, a* values were higher in T1 and T2 than in T0, and in T1 than in T2, the differences being statistically significant.

Between the two batches, statistically significant differences were observed during stage T_1 and T_2 ($p < 0.05$).

3. Assessment of the yellow-blue contrast (b*) indicated statistically significant differences between at least two stages for batch I ($p < 0.001$) and between at least two stages for batch II ($p < 0.05$).

For batches, I and II, the yellow-blue contrast values (b*) were higher in T1 and T2 than in T0, the differences being statistically significant only between T0-T2 and T1-T2.

Between the two batches, there were no statistically significant differences during the three stages ($p > 0.05$).

4. For the shade variation (ΔE*) values, statistically significant differences were observed between at least two variations for batch I ($p < 0.001$), and also for batch II ($p < 0.001$). We made the following abbreviations: ΔE*1 – the difference between T1 and T0, ΔE*2 – the difference between T2 and T0, ΔE*3 – the difference between T2 and T1.

For batch I, ΔE*1 had higher values than ΔE*2 or ΔE*3, the differences being statistically significant ($p < 0.001$). Between ΔE*2 and ΔE*3, the differences were not statistically significant. The same situation was observed for batch II. Between the two batches, there were no statistically significant differences ($p > 0.05$).

5. The statistical analysis for the brightness variation (ΔL*) values revealed statistically significant differences between at least two variations for batch I ($p < 0.01$) and for batch II ($p < 0.01$). We made the following abbreviations: ΔL*1 – the absolute value of the difference between luminosity in stage T1 and luminosity in stage T0, ΔL*2 – the absolute value of the difference between luminosity in stage T2 and luminosity in stage T0, ΔL*3 – the absolute value of the difference between luminosity in stage T2 and luminosity in stage T1. We found statistically significant differences between ΔL*1 - ΔL*3 ($p < 0.001$) in batch I, and statistically significant differences between ΔL*1 - ΔL*2 ($p < 0.001$) in batch II. Between the two batches, there were no statistically significant differences ($p > 0.05$).

6. The statistical analysis for the chroma (C*) values indicated statistically significant differences between at least two stages for both batches I and II ($p < 0.001$). For batch I, chroma (C*) values were higher in T1 and T2 than in T0, the differences being statistically significant between T0-T1, T1-T2 ($p < 0.05$) and T0-T2 ($p < 0.01$). For batch II, chroma (C*) values were also higher in T1 and T2 than in T0, the differences being statistically significant only between T0-T1 ($p < 0.01$) and T0-T2 ($p < 0.05$).

7. The correlation analysis between the values of the studied indicators showed during stage T0, for batch I, a positive correlation between a* and b* and a negative correlation between L* and b*. For batch II we observed a positive correlation between a* and b* and negative correlations between L* and a*, L* and b*, L* and C*.

During stage T1, for batch I we observed positive correlations between L* and b*, a* and b*, L* and C* and a negative correlation between L* and a*. For batch II we observed positive correlations between all the indicators.

During stage T2, for batch I we observed positive correlations between L* and b*, a* and b*, L* and C* and a negative correlation between L* and a*. For batch II we observed a positive correlation between a* and b* and negative correlations between the other indicators.

By applying the original gel obtained experimentally we observed an improvement in all parameters assessing the luminosity and colour, tested teeth turned from a darker shade to a lighter one after the experiment. The mean value for a* was in T1-14.60 and in T2-12.96 for batch I, T1 19.93 and T2 8.76 for batch II, for b* in T1 28.66 and in T2 44.86 for batch I, T1 30.86 and T2 43.50 for batch II, for C* in T1 6.82 and in T2 7.60 for batch I, T1 7.13 and T2 7.26 for batch II and for L* T1 21.30 and in T2 55.30 for batch I, T1 28.20 and T2 59.33 for batch II.

By sequential application of the gel and laser irradiation we obtained beneficial effects, favorable and valid results.

The review of Abdelfattah MM concluded that the use of lasers in teeth whitening is an effective way of bleaching the teeth. There are three dental lasers that have been cleared by Food and Drug Administration for tooth whitening: argon laser, CO_2 laser and diode laser. Other lasers have been tested for teeth bleaching, such as Alexandrite and Nd:YAG.

Another result indicated by Abdelfattah MM is that using the diode laser the enamel's surface was smoother [9].

Son et al. have concluded that teeth brightness was increased by laser assisted whitening, so to achieve the ultimate power bleaching process they used the most effective energy source, in order to avoid any adverse effect. They also showed that by combining the hydrogen peroxide with the diode laser it can not only improve the whitening effect but also protects the changes that take place in the enamel structure compared to the treatment accomplished without laser [10].

The review carried by De Moor et al. showed that from all bleaching wavelengths the diode wavelengths have been the most investigated ones because of the large range of wavelengths that can be used for performing the beaching [11].

The study of Fornaini et al. showed that the variable results on the dental bleaching procedures can be explained by considering that the effects of

whitening depend on various factors, such as the chromophore, the nature of the enamel and the wavelength [12].

Our study brings a plus in the research field because we used an original gel. This research is useful also in the clinical part after testing this gel also on human teeth. Commercial whitening products are very expansive, our original gel once introduced in clinical use will be easy to access for our patients. This is in accordance with the study conducted by Berger et al. [13].

A big number of studies were performed on bovine teeth because it is difficult to obtain human teeth, the source and age of the collected teeth are difficult to be controlled, they are small and have a curved surface that limit some specific tests and also because of the infection hazard. Another problem refers to the ethical requirements of their use. Bovine teeth are easily obtained in large quantities, they are in a good condition and have more uniform composition. The bovine teeth have been suggested as a possible substitute for human teeth for experiments because they have similar chemical and physical properties [14,15].

Yasen et al. showed in his review the advantages of using bovine teeth as a substitute to human teeth [16].

In 2009, Attia et al. demonstrated in an in vitro study that the bovine and human substrates behave similar while undergoing a bleaching process [17].

Further studies are necessary and are being conducted in order to confirm our theses and to define the interaction between the substances used for staining the teeth, the enamel, the gel and it's interaction with the laser.

CONCLUSIONS

Our research demonstrated that the use of chromophore in the experimental gel created by us had a better bleaching effect compared to the same gel without chromophore during laser-assisted bleaching procedures on bovine teeth.

Effects produced by using the lasers depend on two substances, the one that made the coloration and the one with which the bleaching process was made. This experimental trial demonstrates the action of lasers in influencing the bleaching result.

For the a* parameter representing the red-green contrast we observed that the variation of the colour is not depending on the modifications on the red-green axis.

For the b* parameter that gives the value for the yellow-blue contrast for batch I, the batch without chromophore, we did not achieve a significant colour change.

During the measurement of T2 that represented the measurement after the staining process the chromophore prevented the modification of b* according to the whitening gel without chromophore.

The variation of luminosity (L*) is not affected by the presence or absence of the chromophore in the gel. The modification of the parameter luminosity it is due strict to the combination of the whitening gel and the diode laser.

EXPERIMENTAL SECTION

We designed a case control study conducted on bovine teeth. The research was approved by the Ethical Committee of "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj Napoca.

Eight bovine teeth were selected. These types of teeth are very well suited for the spectrophotometric evaluation of colour, because of their large flatness and uniform composition compared to the human teeth.

The extracted bovine teeth were collected from a slaughter house, professionally cleaned (using Satelec P5 Newtron XS) and stored in saline solution.

For the tooth colour measurements we used a Vita Easy Shade Spectrophotometer, that uses the CIEL*a*b* evaluation, model that was proposed by the Commission International de l'Éclerage [18].

The teeth were stained by immersing them for 14 days in a black tea solution that was prepared using three bags of tea 1,7 gram/bag (Lipton Black Tea) in 250 ml of boiling water. After that the shade of the teeth was determined and this measurement represented the T1 value registered the same as for the T0 value with the same spectrophotometer.

Two gel samples were prepared: a reference sample (I) which contained propylene glycol, hydrogen peroxide (38 %), 1.1 % fluoride and 3 % potassium nitrate and a sample (II) which contained additionally methylene blue acting as a chromophore agent.

Both salts, ammonium fluoride (0.11 g) and potassium nitrate (0.3 g) were dissolved in distilled water and a certain concentration of hydrogen peroxide (35 %, ρ=1.13 g/cm^3) solution (3.36 ml) was added for the prepared saline solution. After complete dissolution of the solid component, propylene glycol was added (6.64 ml) to the prepared solution and left to react under magnetic stirring at room temperature for 1 h, until the solution was transformed in a semisolid gel. The gels were prepared by courtesy of dr.Oana Onija.

We divided the teeth in a control (batch I) and a study one (batch II). The teeth from the control batch were kept for 30 sec in the original gel without chromophore and the ones from the study batch in the gel with

chromophore. After that teeth from both batches underwent laser irradiation with continuous wave, using a diode laser (980nm, model Ceralas D15, Ceramoptec, Jena, Germany) for another 30 sec at 50 W.

The shade was determined after the irradiation and the measurement recorder as T2 value as described for the T0 and T1 with the same spectrophotometer.

The difference in color between the different recordings was calculated and obtained by applying the specific formulas [19].

The ΔE^* value represents the shade variation, ΔE^*_1 represents the shade variation between T1 and T0, ΔE^*_2 the shade variation between T2 and T0 and ΔE^*_3 the shade variation between T2 and T1.

The luminosity variation is represented by the ΔL^* value. For the three measurements of the color of the teeth we have ΔL^*_1 representing the absolute value of the difference between luminosity in stage T1 and in stage T0, ΔL^*_2 - the absolute value of the difference between luminosity variation between in stage T2 and in stage T0 and ΔL^*_3 - the absolute value of the difference between luminosity variation between in stage T2 and in stage T1.

All collected values were introduced in an Excel table and all the data collected was statistically analyzed.

Statistical indicators -Elements of descriptive statistics have been calculated and the data was presented using indicators of centrality, location and distribution.

Statistical analysis- Shapiro-Wilk test was used for testing the normal distribution. For data following a normal distribution, Student's *t*-test was used, while for non-normally distributed data, non-parametric tests such as Mann-Whitney (U) or Wilcoxon signed-rank were used. For the analysis of more than two samples, ANOVA or Kruskal-Wallis tests were used. The significance level was $\alpha = 0.05$ (5%), 0.01 (1%) or 0.001.

To measure the statistical dependence between two variables the Pearson (r) correlation coefficient or the Spearman's rank correlation coefficient (rho) were used. For the degree of association we used the Colton empirical rules.

Statistical analysis was performed using StatsDirect v.2.7.2 software, OpenEpi v.3.03 online software and the Excel application (from Microsoft Office 2010 suite).

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