Changes in Bleached Enamel Microhardness After Application of Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) and Diode Laser: An in vitro study

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Tooth bleaching reduces the surface microhardness of enamel. The aim of this study was to evaluate the effect of CPP-ACP paste with diode laser simultaneously on surface microhardness of enamel of bleached teeth. In this in vitro experimental study, forty five healthy anterior teeth of bovine were selected and divided into three groups. Basis microhardness of all the samples was measured by Vickers hardness test. Then all the samples were bleached using 38% H2O2 and their surface microhardness were measured. Then, the first group was treated with CPP-ACP paste, the second group was irradiated with diode laser, and in the third group CPP-ACP+diode laser were used simultaneously. Then the microhardness of enamel was measured again. One-way ANOVA followed by Tukey test was used for statistical analysis. Significance was considered at p<0.05. Tooth bleaching reduced surface microhardness of enamel. The final microhardness of CPP-ACP group has shown significant increase compared to the diode laser group (p=0.001), while microhardness of enamel in the first group did not show significant difference compared to the CPP-ACP + diode laser group (p=0.80). Use of CPP-ACP paste increases microhardness of enamel after bleaching. But use of diode laser associated with CPP-ACP paste does not have any influence on performance of CPP-ACP paste.

Key words: Surface Microhardness, Diode Laser, Tooth Bleaching, CPP-ACP.

Oral health is the most important aspects of health of every human being. Nowadays, beauty is one of the main demands of dental patients. Teeth bleaching is also a conservative treatment of cosmetic dentistry1,2. Various concentrations of substances such as hydrogen peroxide, carbamide peroxide and sodium per borate (with or without heat) are used in the bleaching technique3, 4. Apart from the acceptable esthetic results, many studies have determined that tooth bleaching decreases the enamel’s calcium, phosphate and fluoride content and leads to diminished mechanical properties such as microhardness and strength5.

Diode laser has found numerous applications in dentistry. By increase of temperature, this kind of laser leads to release and emission of positive ions and electrons from the considered surface6. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) which is prepared from milk casein protein contains calcium and phosphate in amorphous form and transfers calcium and phosphate to pellicle which results in a high concentration of calcium and phosphate ions on enamel surface and enhances remineralization7. CPP-ACP application is found in some studies to be the cause of enamel surface microhardness and some studies are stated that its application has no effect on enamel8,9. The aim of the present study was to evaluate the effect of
CPP-ACP and diode laser on enamel microhardness after tooth bleaching.

MATERIALS AND METHODS

In this experimental study, the total numbers of 45 freshly extracted bovine’s incisors without cracks or erosions were cleaned and stored in physiological saline until the beginning of the study. The specimens were divided into three groups of fifteen specimens. Then they were mounted in self-cured acrylic resin so that their labial surfaces were exposed and to be parallel with horizontal plane in order that the required flat surface for microhardness measuring to be provided. Buccal surface of all the samples were polished with diamond polishing discs (FGM, Brazil). Each disc was used only for 5 surfaces. Basic microhardness of all samples of three groups was measured using Vickers hardness test by applying of 500 g force for 15 seconds (MH1, Coopamicrohardness, Iran). The test was performed three times, and then the average of three tests was recorded as the basic microhardness. In the next stage, all samples of three groups were bleached using 38% H2O2 bleaching gel (boost, opalescence) for 15 minutes according to manufacturer’s instructions. The samples were rinsed and dried with air and water spray for 5 seconds. The microhardness of all the samples of each group was measured according to the above method using Vickers hardness measurement test. In this stage the samples of each group were placed under the following three surface preparing methods.

The first group: in this group, CPP-ACP paste (MI Paste GC Corporation, Tokyo, Japan) in uniform thickness of 1 mm was placed on the surface of the samples using applicator. They were rinsed with water for two minutes after passing the basic time of 15 minutes according to manufacturer’s instructions. The samples were placed in distilled water in an incubator at 37°C for 24 hours and then their surface microhardness were measured according to the above method.

The second group: the samples surface was irradiated with diode laser. The radiation protocol was so that diode laser of 810 nm wavelength and at intensity of 1 Watts was irradiated intermittently (Doctor Smile Erbium and Diode Laser, Italy). The process was so that a radiation at intensity of 1 Watts was applied 6 times each for 5 seconds. There was a 5-second rest between two radiations and no radiation applied in this time (rest time is considered to prevent from pulp damage during increase of temperature). Note that according to the above method pulp temperature rise is less than 5°C which cannot be harmful for it. After that the samples were placed for 24 hours in distilled water in an incubator at 37°C and then their microhardness was measured according to the mentioned method for the previous group.

The third group: just like the first group, the surface of the third group was covered with 1 mm of uniform thickness of CPP-ACP paste. Then they were irradiated with diode laser as expressed for the second group. The CPP-ACP paste was rinsed from the surface of the samples after 15 minutes. The samples were placed for 24 hours in distilled water in an incubator at 37°C. Then their microhardness was measured according to the mentioned method for the previous groups.

RESULTS

Statistical analysis was performed with Statistical SPSS software version 21. Kolmogorov-Smirnov test was used to evaluate normal distribution of quantitative data. Because the data had normal distribution, parametric tests were used for data analysis. One-way analysis of variance (one-way ANOVA) and Tukey tests were used for comparison of surface microhardness of bleached teeth enamel in the three groups. Significance set was considered at p<0.05.

The mean and standard deviation surface enamel microhardness for different studied groups have been presented in Table 1. The average of primary microhardness of all teeth was 587.055±148.648 before any bleaching treatment and the average of microhardness of all teeth after bleaching by hydrogen peroxide was 430.842±102.465. The final microhardness of teeth in each group was found to be 494.434±127.017 in total, according to the performed procedure. The average of primary microhardness values after bleaching and in final are listed for each group in the following Table (Table 1).
In multiple comparison of groups each other, no significant difference was observed in the section of primary microhardness. The primary microhardness values of the first group compared to the second and the third groups and also the values of the second group compared to the third group had no significant difference (Table 2).

Table 1. The average of microhardness values of teeth in the three studied groups

<table>
<thead>
<tr>
<th>Groups (Surface Microhardness)</th>
<th>CPP-ACP paste (the first group)</th>
<th>Diode Laser (the second group)</th>
<th>CPP-ACP paste + Diode Laser (the third group)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Microhardness</td>
<td>594.446 ± 169.968</td>
<td>588.598 ± 141.951</td>
<td>577.130 ± 142.205</td>
<td>0.94</td>
</tr>
<tr>
<td>Microhardness after Bleaching</td>
<td>428.084 ± 212.280</td>
<td>429.758 ± 90.904</td>
<td>434.686 ± 97.521</td>
<td>0.98</td>
</tr>
<tr>
<td>Final Microhardness</td>
<td>544.686 ± 142.016</td>
<td>404.130 ± 128.286</td>
<td>534.486 ± 103.766</td>
<td>0.002</td>
</tr>
</tbody>
</table>

No significant difference was observed in the stage of surface microhardness of enamel measurement after bleaching with hydrogen peroxide. So that, no significant difference was observed for microhardness values after application of bleaching in the first group compared to the second and the third groups and in the second group compared to the third group (Table 3).

Table 2. P-values of primary microhardness of groups according to Tukey test

<table>
<thead>
<tr>
<th>Groups</th>
<th>CPP-ACP paste</th>
<th>Diode Laser</th>
<th>CPP-ACP paste + Diode Laser</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPP-ACP paste</td>
<td>-</td>
<td>0.90</td>
<td>0.74</td>
</tr>
<tr>
<td>Diode Laser</td>
<td>0.90</td>
<td>-</td>
<td>0.83</td>
</tr>
<tr>
<td>CPP-ACP paste + Diode Laser</td>
<td>0.74</td>
<td>0.83</td>
<td>-</td>
</tr>
</tbody>
</table>

In the last stage, the microhardness of enamel for the first group in which just CPP-ACP paste was used had significant increase compared to the second group in which diode laser used alone. While the microhardness of enamel for the first group had no significant difference compared to the third group in which CPP-ACP paste was used associated with diode laser. Significant difference was observed in the third group in which two methods of CPP-ACP paste and diode laser were used compared to the second group in which diode laser method was used alone (Table 4).

Table 3. P-values of microhardness after application of bleaching in various groups according to Tukey test

<table>
<thead>
<tr>
<th>Groups</th>
<th>CPP-ACP paste</th>
<th>Diode Laser</th>
<th>CPP-ACP paste + Diode Laser</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPP-ACP paste</td>
<td>-</td>
<td>0.96</td>
<td>0.86</td>
</tr>
<tr>
<td>Diode Laser</td>
<td>0.96</td>
<td>-</td>
<td>0.89</td>
</tr>
<tr>
<td>CPP-ACP paste + Diode Laser</td>
<td>0.86</td>
<td>0.89</td>
<td>-</td>
</tr>
</tbody>
</table>

In the last stage, the microhardness of enamel for the first group in which just CPP-ACP paste was used had significant increase compared to the second group in which diode laser used alone. While the microhardness of enamel for the first group had no significant difference compared to the third group in which CPP-ACP paste was used associated with diode laser. Significant difference was observed in the third group in which two methods of CPP-ACP paste and diode laser were used compared to the second group in which diode laser method was used alone (Table 4).

Table 4. P-values of final microhardness of various groups according to Tukey test

<table>
<thead>
<tr>
<th>Groups</th>
<th>CPP-ACP paste</th>
<th>Diode Laser</th>
<th>CPP-ACP paste + Diode Laser</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPP-ACP paste</td>
<td>-</td>
<td>0.001</td>
<td>0.80</td>
</tr>
<tr>
<td>Diode Laser</td>
<td>0.001</td>
<td>-</td>
<td>0.003</td>
</tr>
<tr>
<td>CPP-ACP paste + Diode Laser</td>
<td>0.80</td>
<td>0.003</td>
<td>-</td>
</tr>
</tbody>
</table>
DISCUSSION

Bovine’s teeth were used in this study. The advantage of use of bovine’s teeth is as follows. It is easier to gather them in large number. Distribution of minerals in bovine’s teeth is similar to human’s teeth, while their structural changes are less than enamel of human’s teeth. In addition, bovine’s teeth have larger flat surfaces. As stated in some studies, 10% carbamide peroxide and 37% hydrogen peroxide bleaching techniques decrease the microhardness of enamel. After bleaching in the current study, enamel microhardness had significant reduction. This result is in accordance with many of previous studies results that even used various concentrations of the substance. However, a study noted that carbamide peroxide did not change enamel microhardness. Difference between the findings of the studies may be a consequence of different concentrations of bleaching agents, different time period for applying of bleaching agents or the type and nature of the tooth itself. Significant increase was observed in final microhardness of the group in which CPP-ACP paste was used for teeth bleaching.

According to study of Bayrak et al. in 2009, use of CPP-ACP and CPP-ACPF paste can increase surface microhardness of enamel after tooth bleaching. Maleki-Pour et al. in 2011 expressed that bleached samples in which fluoride and CPP-ACP was used, had more surface microhardness than untreated bleached samples. This finding was statistically significant. No significant difference was observed between fluoride and CPP-ACP. Yamaguchi et al. in 2009 found that the amount of microhardness in demineralized teeth of abovine increased after being exposure to CPP-ACP paste and regarding the evaluation of the effect of this substance on the enamel of the calf, they showed that there is high concentration of inorganic components in the CPP-ACP paste that increases remineralization of tooth enamel. Surface microhardness of teeth also was increased by CPP-ACP paste in the current study. One of the considered explanations for that could be the impact of CPP-ACP paste on reduction of demineralization and increase of remineralization. CPP has an important role in transfer of ACP and in localization of large amount of soluble calcium and phosphate. The localization maintains the slope of calcium and phosphate concentration at the surface of tooth enamel and thus facilitates remineralization.

No significant increase in surface microhardness of enamel was observed in the teeth that were only irradiated by diode laser. Balabuc et al. in 2007 reported an increase in surface microhardness of enamel after irradiating with 980 nm diode laser. Microfusion of enamel and change in morphology were mentioned by them as the cause of this phenomenon. Difference in the used power can be considered as the cause of difference between the results of the current and Balabuc studies. No study has found to investigate 810 nm diode laser’s effect on microhardness of tooth.

Although in the third group of the current study in which CPP-ACP + laser were used, the amount of final microhardness was more than the laser group. This difference is significant, while it did not have a significant difference with CPP-ACP group. In other words, the effectiveness of CPP-ACP paste in increase of surface microhardness of enamel was not change with diode laser radiation.

Vitale et al. performed a study to evaluate effect of diode laser on fluoride absorption of enamel. They found that diode laser increases the amount of fluoride absorption by tooth enamel. It was stated in the study of Gonzalez-Rodriguez et al. in 2011 that diode and CO₂ lasers increase the amount of fluoride absorption by enamel of tooth. Various studies showed that diode laser increases the amount of fluoride absorption by enamel of tooth. However, no study has found to investigate the effect of CPP-ACP paste associated with diode laser on surface microhardness of enamel.

CONCLUSIONS

It can be concluded according to the obtained results of the current study that use of CPP-ACP paste increases microhardness of teeth after bleaching. Diode laser alone does not have any effect on final microhardness of teeth and it does not have any influence on efficacy of CPP-ACP paste in increase of enamel microhardness after bleaching.
REFERENCES


