The Effect of Photodynamic Therapy and Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) on the Remineralization Rate of Non-Cavitated Root: an In-vitro Study

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ABSTRACT

Using laser treatments and calcium and phosphate compounds to enhance remineralization has been investigated in this study. Seventy two premolar teeth were divided into four groups of 18: 1) control group; 2) laser therapy group; 3) CPP-ACP paste group; and 4) laser therapy and CPP-ACP group. Mineralization and remineralization of samples were investigated by Diagnodent. Data were reported using descriptive statistics (mean, standard deviation) and One Way ANOVA; they were analyzed using SPSS.16 statistical software. Statistical analysis showed that groups 3 and 4 had the highest rate of remineralization compared to groups 1 and 2. According to the results of this study, mineralization ranged decreasingly from group 4 to groups 3, 2, and 1, respectively. The antibacterial effect of laser therapy, leading to remineralization of calcium and phosphorus compounds, was the most effective on controlling root decay.

Keywords: photodynamic therapy, CPP-ACP, root caries, tooth remineralization.
INTRODUCTION

One of the problems faced by patients with gingival recession is root exposure, which causes root decay due to low resistance of semen to demineralization. Caries are caused by the replacement of a number of bacteria capable of demineralization. In neutral conditions, hydroxyapatite is in equilibrium with calcium and phosphorus rich saliva, but when the pH is reduced below 5.5, hydrogen ions produced by bacteria react with the phosphate group of enamel crystals and convert \( \text{PO}_4^{2-} \) into \( \text{HPO}_4^{2-} \) that cannot further form the crystalline net properly. Nevertheless, caries can be reversed in neutral pH and presence of calcium and phosphorus during a process called remineralization (1).

Root caries induce sensitivity to sweets and cold and disturb patients (2). Also, due to exposure of cement during brushing, it may be eroded due to the softness of the cement (3).

One of the methods to increase remineralization is CPP-ACP (Casein Phosphopeptide-Amorphous Calcium Phosphate), which results from decomposition of milk proteins and their combination with calcium and phosphorus. Casein phosphopeptide consists of ser (p)_ser (p)_ser (p) Glu_Glu phosphoseryl segments, by which calcium is bound to ACP. In the oral area, ACP is separated from CPP, enters the denture plaque and buffers the activity of calcium ions, providing an environment saturated by calcium and phosphorus, and reforms hydroxyapatite that increases remineralization and decreases demineralization (1, 4). Also, CPP-ACP reduces tooth sensitivity and erosion (3, 5), and its use has been proven to stop caries in both in vivo and in vitro studies (6).

Photo-activated disinfection (PAD) is another new increasingly used method nowadays, in which free oxygen radicals that are produced by Yer laser radiation on a photo sensitizer selectively destroy the target cells (here, bacterial cells) and decay-inducing bacteria (7, 8).

Jayarajan et al investigated the effect of CPP-ACP and CPP-ACP (combination of CPP-ACP and fluoride) on enamel remineralization in vitro and concluded that both materials increased remineralization of dental caries, with CPP-ACP being more effective than CPP-ACP (4).

Bonstein et al examined the effect of PAD use in reducing the number of Streptococcus intermedius in dentinal samples in vitro and concluded that PAD was effective in reducing the number of bacteria in these samples (9).

J. P. Rolim and colleagues investigated the effect of PAD and CPP-ACP on root remineralization in a case study and found that the two methods were more effective in increasing root canal remineralization when used simultaneously than separately (7).

Considering that a comprehensive study to explore the simultaneous effect of PAD and CPP-ACP on root canal remineralization has not been done yet, and because the studies of Jayarajan et al and Rolim, who simultaneously examined the two substances, were case reports, we decided to investigate the effect of simultaneous use of the two methods in non-cavitated root in addition to that of separate treatment with PAD and ACP-CPP.

MATERIALS AND METHODS

According to the study by Vlacic et al (2), the mean number of Diagnodents was considered 5.31 in evaluation intervals for the CPP-ACP group, taking into account a 10% Diagnodent value in different groups and a standard deviation of 1.5 units in Diagnodent number, and considering \( \alpha = 0.05 \) and a power of 80%, a sample size of 72 was estimated. All samples were selected by convenient sampling from a number of mandibular premolar teeth that had no white spots, caries and cracks on physical and microscopic examination (Nikon, Japan). After assessing the amount of demineralization available by Diognodent, they were randomly divided into four groups: control (1), PAD (2), CPP-ACP (3), and simultaneously used CPP-ACP and PAD (4). In all groups, the amount of remineralization was measured by Diognodent.

Seventy two mandibular premolar teeth were selected by convenient sampling and debris, plaque, and soft tissue were removed and placed in 0.5% chloramine solution. After separating the crown and roots of all samples to evaluate the process of demineralization, coronal Diagnodent 1.3 with buccal root (Kavo Dental NC, Germany) was used. For this purpose, Diagnodent (fissural), which had been initially calibrated according to the manufacturer and based on each tooth, was...
used to measure the amount of demineralization at three or more points near the tooth surface. Afterwards, means of the values were recorded, and teeth showing numbers 3-7 (which indicated intactness of the enamel) were selected (Figure 1). A 4*4 mm paper was attached to the coronal part of the buccal root of each sample (to limit the study field) and put in Acid Resistance Nail Varnish. After paper removal, samples were washed with deionized water (4) and kept in a caries-inducing medium containing Heart-Brain Infusion broth with 107 CFU/mL of Strep­tococcus mutans (IBRC-M 10682) and Lactoba­cillus acidophilus (IBRC-M NO = 10815) (from the National Center for Genetic and Biochemical Reserves) and artificial saliva (20 mmol/L NaHCO3 mmol/L NaH2PO4 and 1 mmol/L CaCl2) and incubated at 37°C; the amount of demineralization was measured by Diagnodent for each sample. When the 72 samples showed the number of 9, incubation was stopped. Then, samples were washed and dried with deionized water and dried and kept in an artificial saliva containing 107 CFU/mL of Streptococcus mutans and Lactobacillus acidophilus (at room temperature, neutral pH, and stable air flow) (4). Afterwards, samples were divided into four groups of 18. The first group (control) was kept only in artificial saliva, and no substance was used.

In the second group, PAD Reimers Janssen and GmbH Germany (RJ-LASER) was used; for this purpose, 12.7 mg/L tolonium chloride was applied to the desired location with an applicator and radiated for 1 min with a laser of 670-690 nm wavelength, 8-10 μm/cm² intensity and 0.5 watts power. Then, samples were washed with deio­nized water daily for seven days and the amount of remineralization was evaluated at the end of each day with Diagnodent.

In the third group, CPP-ACP (GC TOOTH-MOUTH 76-1 Hasunuma-cho, Itabashi-ku, To­kyo 174-8585, Japan) was used. The CPP-ACP substance was applied to the studied area for four minutes every 24 hours and continued for seven days, then it was rinsed with deionized water and returned to the artificial saliva. At the end of each day, the amount of existing remineralization was measured (4).

In the fourth group, PAD and CPP-ACP were concomitantly used. For this purpose, PAD was first used in the same way as for group 2. Then, samples were washed and CPP-ACP was used at the desired site similarly to group 3, and this simultaneous use of PAD and CPP-ACP was performed every 24 hours for seven days. At the end of each day, the amount of existing remineralization was measured.

![FIGURE 1. A) Measurement of caries in samples by the Diagnodent device; B) Laser therapy in one of the study groups](image-url)

![FIGURE 2. Comparison of mean demineralization between study groups](image-url)
Finally, data were entered into SPSS 16 software and analyzed. Results were reported as mean ± SD. To test the normal distribution of variables, Kolmogorov-Smirnov test was used. In order to compare the differences among the four groups, one way ANOVA, and subsequently Tukey post hoc test were used. P < 0.05 was considered as significant level.

RESULTS

The descriptive statistics of the studied groups are shown in Table 1 and Figures 1 and 2. There was a significant difference between the groups based on one way ANOVA test. Tukey post hoc test showed that there was a significant difference among the four groups: between CPP-ACP paste and laser (p < 0.0001), CPP-ACP laser and paste-laser (p < 0.0001), CPP-ACP paste and CPP-ACP laser-paste (p < 0.0001), control and CPP-ACP paste (p < 0.0001), and control and CPP-ACP laser-paste (p < 0.0001). This test showed that there was no significant difference between control and laser groups (p = 0.99).

DISCUSSION

In the oral environment, the tooth structure is placed under demineralization and remineralization conditions. If this equilibrium collapses and demineralization progresses, tooth structure is destroyed due to bacterial activity and acid production (10, 11). Different studies have suggested different methods for disturbing this equilibrium. The use of fluoride compounds and compounds containing calcium and phosphate groups is one of the most commonly used materials (12).

Today, remineralization is an important research topic, but it is still difficult to pinpoint the effectiveness of various methods of remineralization. In this study, the effect of several methods to stop the smooth lesions of root surfaces has been investigated; lesions were created using Streptococcus mutans and Lactobacillus acidophilus bacteria in order to simulate the clinical conditions as much as possible and to investigate the processes more precisely. Borisova et al studied the enhancing effect of autofluorescence of caries using laser and induced decay by bacteria (13).

In this study, the decay activity was monitored and evaluated by Diagnodent, a laser device showing decay activity numerically at a wavelength of about 500 nm. In a study by De Queiroz et al, the impact of fluoride and diet on remineralization was studied and demineralization of dental tissues around orthodontic brackets and Diagnodent was used for measurement of demineralization (15).

In this study, photodynamic therapy was employed to remove acid bacteria in active root decay. The reason for using a wavelength of 670-690 nm was the absorption ability in toluidine chloride photo sensitizer, an available material that is not toxic to the tissues.

In groups 2 and 4, that received laser treatment, TBO (Toluidine Bloworta) was used as a photo sensitizer. Rolim et al reported that TBO had the strongest effect on reduction of Streptococcus mutans compared to other photo sensitizers, most likely due to the TBO absorption range of about 630 nm, very close to the absorption wavelength of 660 nm used by researchers (7).

In the present experimental study, which investigated the effect of photodynamic therapy

<table>
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<th>Group</th>
<th>N</th>
<th>Mean±SD</th>
<th>Minimum</th>
<th>Maximum</th>
<th>P-value</th>
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<tr>
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<td>43.00</td>
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<tr>
<td>CPP-ACP &amp; Laser (PDT)</td>
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<td>18.35±6.42204</td>
<td>7.00</td>
<td>28.00</td>
<td></td>
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<td>31.50±11.00000</td>
<td>7.00</td>
<td>54.00</td>
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</table>

TABLE 1. Comparison of mean demineralization between study groups
and CPP-ACP on the rate of non-cavitated root canal remineralization, results showed that the use of photodynamic therapy and CPP-ACP had a significantly higher impact on enhancement of remineralization comparatively to controls and other methods; also, CPP-ACP had the highest remineralization rate. There was also no significant difference between the control group and the photodynamic therapy group in terms of remineralization rate.

In this study, there was a significant difference between group 3 (CPP-ACP paste) and the control group. Jayarajan et al. showed that the use of CPP-ACP paste increased teeth remineralization due to the mineral supply required for teeth. In the oral environment, ACP is separated from CPP and enters the dental plaque, which buffers the activity of calcium and phosphate, causing remineralization (4).

Comparison showed a significant difference between CPP-ACP paste and laser groups (photodynamic therapy) (groups 3 and 2), but not also between control and laser groups (groups 1 and 2, respectively); however, in the study of Rolim et al., the use of TBO and MG was more effective in reducing Streptococcus mutans than control group (7). This can be due to the fact that laser use reduces the number of Streptococcus mutans and stops demineralization. However, in this method, minerals required for remineralization were not available to the teeth, explaining why there was no significant remineralization. This can justify the important difference between groups 3 and 4, where laser provides the remineralization material.

When compared to group 3, group 4 showed a statistically significant difference and a higher remineralization rate than the other groups. Laser use reduces the number of Streptococcus mutans (15), limiting the loss of mineral material, and CPP-ACP paste also increases the rate of remineralization by supplying minerals to the tooth (2). In the study of Vlacic et al., the simultaneous use of laser and paste of CPP-ACP increased teeth remineralization significantly compared to the primary state of the teeth (2). Fekrazad et al. reported that the simultaneous use of Radachlorin and laser was more effective in reducing Streptococcus mutans than laser alone (16). According to Poggio et al., the antibacterial properties of PAD were enhanced by using 5% NaOCl (17). These findings suggest that laser may have synergistic effects by reducing bacterial activity and CPP-ACP by providing calcium and phosphorus sources.

The results of this study were similar to those using CPP-ACP to increase remineralization but so far, no original study has evaluated the effect of laser and CPP-ACP paste; also, only case studies have been published, and their results were consistent with ours.

**CONCLUSION**

According to the results of this study, it can be concluded that the simultaneous use of photodynamic therapy and CPP-ACP can increase remineralization compared to controls and other remineralization methods. Also, there was no significant difference between control group and photodynamic therapy group in terms of remineralization.

**Conflicts of interest:** none declared.

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**REFERENCES**


