Effect of Er,Cr:YSGG Laser and CPP-ACP on the Prevention of Enamel Demineralization (In Vitro Study)

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ABSTRACT:

Objectives: This study was conducted to compare the effect of Er,Cr:YSGG laser, Casein Phosphopeptide - Amorphous Calcium Phosphate (CPP-ACP), and the combined effect of their use on the prevention of enamel demineralization in a sample of extracted premolars. Firstly, using stereomicroscope to assess the severity of the White Spot Lesions (WSLs) visually and secondly, using polarized light microscope to assess the lesions depth.

Materials and Methods: Eighty sound extracted premolar teeth were randomly allocated to four equal groups (n=20). Group I: Control group received none of the preventive treatments. Group II: CPP-ACP topical application. Group III: Er,Cr:YSGG laser irradiation. Group IV: Er,Cr:YSGG laser irradiation followed by CPP-ACP application. Specimens were subjected to thermocycling and brushing challenge protocols equivalent to 1 year in oral environment. Then, all teeth were subjected to acid challenge. Each tooth was examined visually and photographed using stereomicroscope to assess the severity of the WSL. The teeth were then sectioned longitudinally and examined under polarized light microscope and the depth of the lesions were measured and compared between the four groups.

Results: The combined use of laser and CPP-ACP (Group IV) resulted in the formation of the least severe WSLs and the least lesions depth when compared to the control group. CPP-ACP alone and laser alone also showed a significant difference in the severity of WSLs and lesion depth when compared to the control group, however, no significant difference was found between them. Conclusions: The combined use of Er,Cr:YSGG laser and CPP-ACP showed the best prevention against the development of WSLs regarding the severity and depth. The use of CPP-ACP alone or laser alone also resulted in a significant reduction in lesion depth and severity but was significantly less than their combined use. No significant difference was found between the CPP-ACP alone and laser alone groups.

Keywords: Er,Cr:YSGG laser, CPP-ACP, White spot lesions, enamel demineralization, Prevention.

INTRODUCTION

Patients commonly pursue orthodontic treatment to enhance esthetics.¹² However, treatment with fixed orthodontic appliances can result in the formation of White Spot Lesions (WSLs) on the labial and buccal surfaces of the teeth thus generating anotheresthetic concern for the patient and triggering disappointment for both the patient and the orthodontist at the time of debonding.³ Fixed orthodontic appliances increase the number of plaque retention locations in otherwise unsusceptible areas of the teeth.⁴⁵

The demineralized enamel is more porous in comparison to sound enamel and the crystal structure is partially substituted with water, with a difference in the refractive indices causing a larger degree of light scattering.

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in demineralized enamel. Consequently, initial enamel demineralization expresses itself clinically in the form of WSLs, where the surface of demineralized enamel seems whiter in color than sound enamel.\(^{(6,7)}\)

WSLs can develop quickly in the presence of plaque. Studies have revealed that clinically visible lesions may be detected as early as 4 weeks after bonding or banding.\(^{(5,8)}\) Therefore, the prevention of plaque accumulation by applying proper oral hygiene measures, including tooth brushing with a fluoridated toothpaste and the use of fluoride mouth rinses, is essential for controlling the development of WSLs.\(^{(9-11)}\) However, regardless of the usage of prophylactic measures, Hadler-Olsen et al.\(^{(12)}\) stated that 60% of the orthodontic cases developed one or more new WSLs at the termination of orthodontic therapy.

Various commercially available dental materials can assist in the prevention and remineralization of WSLs, such as materials containing Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) and calcium sodium phosphosilicate or bioactive glasses.\(^{(13)}\) Casein phosphopeptide produces nanoclusters with amorphous calcium phosphate hence providing a pool of calcium and phosphate which can preserve the super saturation of saliva. As CPP-ACP is able of stabilizing the calcium and phosphate in the solution, it can likewise aid in the buffering of plaque pH, thus calcium and phosphate plaque level is raised. As a result, calcium and phosphate concentration within the subsurface lesions is maintained high which results in reducing demineralization and enhancing remineralization of enamel lesions.\(^{(13)}\) Several researches have demonstrated the beneficial caries-preventing and enamel remineralization effect of CPP-ACP.\(^{(14-18)}\)

In the past years, it has been documented that treatment with numerous types of lasers can decrease the rate of subsurface demineralization in enamel and a number of justifications have been given for this process including: enamel surface melting, recrystallization, decrease in enamel permeability after laser irradiation as well as altering the enamel organic matrix, but still the exact mechanism is unclear.\(^{(19-22)}\)

The commonly used lasers for caries prevention on enamel are Nd:YAG; CO\(_2\); Er,YAG; Er,Cr:YSGG; and Argon.\(^{(19-27)}\) To accomplish additional prevention of enamel demineralization, special attention has concentrated on laser irradiation in conjunction with topical fluorides and remineralizing solutions.\(^{(19-26,28)}\) Numerous former studies supported the finding of increased enamel acid resistance using laser irradiation in combination with fluoride through their synergistic effect displaying more favorable results.\(^{(19,22,23,26)}\)

Up to our knowledge, this study was the first in the literature to evaluate the effect of the combined use of Er,Cr:YSGG laser irradiation and CPP-ACP topical application on human permanent teeth. The null hypothesis was that there will be no significant difference in the prevention of enamel demineralization when combining Er,Cr:YSGG laser irradiation with CPP-ACP topical application in comparison to either laser irradiation alone or CPP-ACP topical application alone.

**MATERIALS AND METHODS**

A randomized controlled in vitro study design was used to address the aim of the study. The sample size estimation was calculated using Power and Sample Size Calculations computer software (Epi-Info 7 soft wares). At \(\alpha= 0.05\) and a power of 0.80, a minimum sample size of 15 teeth per group was required.\(^{(29,30)}\) It was decided to collect 20 premolars for each group for a total sample size of 80 premolars.

Eighty human premolars, freshly extracted for orthodontic purpose were collected from subjects treated in the Department of Orthodontics, Faculty of Dentistry, Alexandria University. All the subjects were born and resided in Alexandria, Egypt, where the average level of fluoride in the drinking water is 0.61 ppm and no additional fluoride is added to the drinking water.\(^{(31)}\)

All the selected teeth had intact buccal enamel surface, with no obvious decalcifications, caries,
cracks or stains. Additionally, the teeth were not previously bonded or received any chemical treatment.

1-Teeth preparation

Following extraction, all soft tissue remnants, calculus and other extraneous materials were removed and the teeth were cleansed with tap water and stored in artificial saliva solution (20 mmol/l NaHCO₃, 3 mmol/l NaH₂ PO₄, 1 mmol/l CaCl₂) at 37°C for the duration of the study with a neutral pH 7 to simulate the oral environment. The solution was changed every day.

Upon the beginning of the experiment, all teeth were cleansed and polished with rubber prophylaxis cup using a low speed hand piece with a mixture of non-fluoridated, oil free pumice and water and then rinsed with running water for 10 seconds.

Each tooth was assigned a number from 1 to 80 for identification purposes and these numbers were used to randomly allocate the samples to the four study groups. A permanent marker was used to give each tooth an identification number on the root facilitating teeth distribution to their belonging groups. All teeth were washed under running water for 60 seconds and dried with moisture and oil free jet of air to be examined visually and photographed by the stereomicroscope at the baseline.

2- Grouping

The teeth were randomly assigned to the four groups of 20 each using a random number generator and each group was stored in a separate beaker labeled with the group name and containing 200 ml of artificial saliva solution at room temperature.

Group I (Control group):

The teeth received no preventive measures before the induction of WSLs.

Group II (CPP-ACP group):

Teeth in this group received CPP-ACP topical application (GC Tooth Mousse-GC International, Itabashi-Ku, Tokyo, Japan) according to the manufacturer’s instructions. It was applied on the whole surface and left undisturbed for 5 minutes. This was repeated at the same time of the day for a period of 5 days.

Group III (Er,Cr:YSGG laser group):

In this group, the teeth received Er,Cr:YSGG Laser irradiation on enamel performed using an Er,Cr:YSGG device (Water Lasei Plus™, Biolase Inc., USA). This Laser emits photons at wavelength of 2.78 µm (Figures 1, 2).

Sub-ablative irradiation conditions were applied based on previous studies where the power was 0.25 W, pulse energy was 12.5 mJ, energy density was 8.5 J/cm², frequency was 20 Hz, and pulse duration was 140 microseconds applied for 20s. A G6 tip was used with a diameter of 600 mm and 6 mm length in noncontact focused mode. The handpiece was held perpendicular to enamel surface and the samples were irradiated once in each direction moving slowly horizontally and vertically in order to promote homogenous irradiation and to cover the entire sample area in a scanning style with 11% air and no water cooling system (Figures 4).

Group IV (Combined Er,Cr:YSGG laser and CPP-ACP group):

Teeth were lased by Er,Cr:YSGG laser with the same sub-ablative conditions mentioned above, followed by CPP-ACP topical application (GC Tooth Mousse) according to the manufacturer’s directions.

3- Simulation of oral environment

To simulate normal oral environment, teeth were subjected to brushing and thermocycling protocols. All teeth groups underwent 9 separate rounds of 800 brush strokes and thermocycling for 110 cycles with a total of 7200 brush strokes and 1000 thermocycles which is equivalent to oral environment for 1 year.

The specimens were stabilized in the toothbrush machine mounts using putty viscosity rubber base material (SwissTec, Coltène/Whaledent Inc., Ohio, USA) mold, to facilitate easy removal of the specimens for the thermocycling and remounting again for the subsequent brushing cycles.
The mounted teeth were brushed using brushing machine with soft bristled brushes centered over the buccal surfaces of the teeth with a constant force applied to each brush. Slurry of non-fluoridated toothpaste and water (1:3 ratio) was constantly recirculated by the machine. Brushes heads were changed after each cycle.  

Thermo cycling protocol was executed by cycling the specimens in water between 5°C and 55°C with a dwell time of 20 seconds and transfer time of 5 seconds.

4- Acid challenge (WSLs induction)

The teeth were stored in artificial saliva solution, and then placed in standard Ten Cate demineralizing solution at pH 5 consisting of 2.20 mM Calcium, 2.20 mM Phosphate, 50 mM buffer (acetic acid/ K acetate) at 37°C for 1 hour every 11 hours. Teeth were cycled between artificial saliva and Ten Cate demineralizing solutions for 35 days to perform the acid challenge and induce the WSLs. The solutions were changed every other day during the experiment, and each group was cycled in separate beakers of solutions throughout the experiment.

5- Stereomicroscope examination

Teeth were washed under running water for 60 seconds and dried with moisture and oil free jet of air for 15 seconds. Each tooth was examined visually and photographed using stereomicroscope, and a scoring system was used to assess the severity of the WSLs as follows:

0= No visible white spots
1= Slight visible white spots
2= Excessive visible white spots
3= White spots with cavitation

6- Specimens preparation for polarized light microscope examination

The teeth were sectioned longitudinally in a bucco-lingual direction using a diamond disk under water cooling, and then tooth material
was reduced by hand grinding on polishing boards, using progressively finer grades of aluminum oxide powder until a thickness of 150 µm was reached.\cite{43,45}

The sections were washed with deionized water and oriented longitudinally on glass cover slides. The sections were photographed using polarized light microscope under maximum illumination. Photomicrographs were made at 50X magnification. The polarized light microscope is connected to a computer with an imaging analysis software program having a digital ruler that can measure all over the lesion recording the largest and the smallest lesion depths, and then the average lesion depth was calculated accordingly. The lesion depth was calculated in millimeters using a 1:50 scale and then converted into µm by multiplying it by $10^3$.

The steps of the study are illustrated in Figure 5.

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**Figure 5:** Flow chart of the study methodology.
**STATISTICAL ANALYSIS**

The data was fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using number and percentage. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level. The used tests were: Chi-square test: for categorical variables, to compare between different groups; Fisher’s Exact or Monte Carlo correction: for correction for chi-square when more than 20% of the cells have expected count less than 5; Kruskal Wallis test: for abnormally distributed quantitative variables to compare between more than two studied groups; and Post Hoc (Dunn’s multiple comparisons test): for pairwise comparisons.

**RESULTS**

The present study aimed at evaluating the effect of using Er,Cr:YSGG laser, CPP-ACP topical application, and their combined use on the prevention of enamel demineralization by assessing the severity of WSLs visually using stereomicroscope and by assessing the lesion depth using polarized light microscope.

### A. **Stereomicroscopic examination of the samples:**

Stereomicroscopic examination of the samples was done at the beginning of the study (baseline) and after induction of the artificial WSLs. Comparison of the severity of the WSLs between the four studied groups is shown in Table 1 and Figure 6. Figures 7-11 represent stereomicroscopic pictures of a tooth sample at baseline and after induction of the WSL from each studied group.

**Table 1: Comparison of the severity of the WSLs between the four studied groups.**

<table>
<thead>
<tr>
<th>Severity of lesion by scoring system</th>
<th>Group I (n = 20)</th>
<th>Group II (n = 20)</th>
<th>Group III (n = 20)</th>
<th>Group IV (n = 20)</th>
<th>$\chi^2$</th>
<th>MC $p$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>20(100.0%)</td>
<td>20(100.0%)</td>
<td>20(100.0%)</td>
<td>20(100.0%)</td>
<td>77.031*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>1</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>After testing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0(0.0%)</td>
<td>10(50.0%)</td>
<td>8(40.0%)</td>
<td>18(90.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0(0.0%)</td>
<td>9(45.0%)</td>
<td>9(45.0%)</td>
<td>2(10.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4(20.0%)</td>
<td>1(5.0%)</td>
<td>3(15.0%)</td>
<td>0(0.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>16(80.0%)</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Significance between groups</strong></td>
<td>$\text{MC}p_1&lt;0.001^<em>$, $\text{MC}p_2&lt;0.001^</em>$, $\text{MC}p_3&lt;0.001^<em>$, $\text{MC}p_4=0.732$, $\text{MC}p_5=0.013^</em>$, $\text{MC}p_6=0.003^*$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$\chi^2$, $p$: $\chi^2$ and $p$ values for Chi-square test for comparing between the four groups.

$\text{MC}p$: $p$ value for Monte Carlo for Chi-square test for comparing between the four groups and each two groups.

$p_1$: $p$ value for comparing between group I and group II.

$p_2$: $p$ value for comparing between group I and group III.

$p_3$: $p$ value for comparing between group I and group IV.

$p_4$: $p$ value for comparing between group II and group III.

$p_5$: $p$ value for comparing between group II and group IV.

$p_6$: $p$ value for comparing between group III and group IV.

*: Statistically significant at $p \leq 0.05$. 

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At the baseline, all teeth in all 4 groups were scored 0 as teeth were selected and included in the study based on having intact enamel surface with no WSLs.

After induction of the WSLs, the control group (Group I) had 16 teeth that scored 3 showing WSLs with cavitation and 4 teeth were scored 2 showing excessive WSLs formation. On the other hand, the CPP-ACP group (Group II) showed statistically significant difference in the severity of WSLs formation when compared to the control group having 10 teeth with no visible WSLs, 9 teeth scored 1 showing slightly visible WSLs, only 1 tooth with excessively visible WSLs and no teeth in this sample showed WSLs with cavitation.

Regarding the Er,Cr:YSGG group (Group III), it showed statistically significant difference in the severity of WSLs from the control group, and higher severity but statistically insignificant difference when compared to the CPP-ACP group. Eight teeth showed no visible WSLs formation, 9 teeth scored 1 showing slightly visible WSLs, 3 teeth showed excessively visible WSLs and again no teeth in the sample showed WSLs formation with cavitation.

Finally, the combined use of Er,Cr:YSGG laser and CPP-ACP (Group IV) showed a statistically significant difference in WSLs severity when compared to all other 3 groups where 18 teeth scored 0 with no visible WSLs formation, only 2 showed slightly visible WSLs and no teeth in this group showed either excessive WSLs formation nor cavitation.

**B. Polarised light microscope examination:**

Polarised Light Microscope (PLM) examination was done to quantitatively measure the demineralized lesion depth between the studied groups.

**1- Histological evaluation using the PLM pictures:**

All the sections displayed increased birefringence (double refraction) of the enamel when examined under the PLM. The positively birefringent zone was located beneath a narrow negatively birefringent surface layer.
Figure 7: Premolar at baseline showing intact buccal surface.

Figure 8: Premolar from CPP-ACP group showing slightly visible WSLs.

Figure 9: Premolar from laser group showing slightly visible WSLs with surface roughness.

Figure 10: Premolar from combined laser and CPP-ACP group showing no visible WSLs formation.

Figure 11: Premolar from the control group showing excessively visible WSLs formation with cavitation and brown discoloration.

Group I

The positively birefringent zone in the sections of the control group appeared more homogenous than the other three groups, and was covered by a thin negatively birefringent surface layer. The lesions extended deep into the enamel layer before merging with the normal birefringence of intact enamel (Figure 12).

Group II

Lesions observed in sections of the CPP-ACP group showed areas of increased birefringence which appeared less homogeneous and less deep than the control group (Figure 13).

Group III

Lesions observed in the irradiated Er,Cr:YSGG laser group showed areas of
increased birefringence which appeared less homogeneous and less deep than the control group but deeper than the CPP-ACP group (Figure 14).

**Group IV**

Sections of the combined use of Er,Cr:YSGG laser and CPP-ACP group showed areas of normal enamel birefringence extending between areas of increased birefringence of the lesions. In addition, they showed more superficial merge of the subsurface lesions with the normal enamel compared to the other groups. It also showed the least lesion depth when compared to all other three groups (Figure 15).

2- Evaluation of the depth of demineralized lesions using the PLM pictures:

A comparison of the mean depth of demineralization in µm among the study groups is shown in Table 2 and Figure 16.

Upon simulation of the oral environment equivalent to one year, the control group showed the deepest lesion depth (328.56 µm ± 127.99 µm) followed by the Er,Cr:YSGG laser group (180.45 µm ± 24.27 µm), then the CPP-ACP group (168.48 µm ± 28.84 µm), and the least lesion depth was found in the combined use of Er,Cr:YSGG laser and CPP-ACP group (24.5 µm ± 8.6 µm).

The CPP-ACP group and the Er,Cr:YSGG laser group resulted in 48.7% and 45% less lesion depth respectively when compared to the control group. A statistically significant difference was found when comparing each group of them to the control group, however no statistically significant difference was found between the CPP-ACP group alone and the Laser group alone.

On the other hand, the combined use of Er,Cr:YSGG laser and CPP-ACP resulted in 92.5% less lesion depth when compared to the control group. A statistically significant difference was found between group IV and all the other three groups.

![Figure 12: PLM picture of a section in the control group showing positive birefringence extending deep into the enamel before merging with normal enamel birefringence.](image1)

![Figure 13: PLM picture of a section in the CPP-ACP group showing less homogeneous areas of increased birefringence and a less deep lesion than control group.](image2)

![Figure 14: PLM picture of a section in the laser group showing areas of increased birefringence which appeared less homogeneous and less deep than the control group but deeper than the CPP-ACP group.](image3)

![Figure 15: PLM picture of a section in the combined use of laser and CPP-ACP group showing more superficial merge of the subsurface lesions with the normal enamel compared to other groups with the least lesion depth.](image4)
Table 2: Comparison between the four studied groups according to lesion depth.

<table>
<thead>
<tr>
<th>Lesion depth (µm)</th>
<th>Group I (n = 20)</th>
<th>Group II (n = 20)</th>
<th>Group III (n = 20)</th>
<th>Group IV (n = 20)</th>
<th>H</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. – Max.</td>
<td>98.8 – 492.9</td>
<td>122.5 – 199.7</td>
<td>131.1 – 206.8</td>
<td>11.4 – 33.1</td>
<td>53.243*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>328.56±127.99</td>
<td>168.48 ± 28.84</td>
<td>180.45 ± 24.27</td>
<td>24.5 ± 8.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>360.2</td>
<td>174.65</td>
<td>186.15</td>
<td>28.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance between groups</td>
<td>p₁=0.004*, p₂=0.044*, p₁&lt;0.001*, p₄=0.384, p₅&lt;0.001*, p₆&lt;0.001*</td>
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</table>


Significance between groups was done using Post Hoc Test (Dunn's multiple comparisons test).

p₁: p value for comparing between group I and group II
p₂: p value for comparing between group I and group III
p₃: p value for comparing between group I and group IV
p₄: p value for comparing between group II and group III
p₅: p value for comparing between group II and group IV
p₆: p value for comparing between group III and group IV

*: Statistically significant at p ≤ 0.05.

Figure 16: Bar graph showing comparison of the mean lesion depth between the four studied groups.
DISCUSSION

Fixed orthodontic therapy has been reported to increase the prevalence of WSLs on the labial and buccal surfaces of teeth regardless of the use of prophylactic measures. Oggaard showed that even 5 years after treatment, orthodontic patients had a significantly higher incidence of WSLs than a control group of patients who did not receive treatment.

A paradigm shift is emerging in dentistry targeting maximum conservation of tooth structure. Many preventive measures have been described in the literature in order to increase enamel resistance to demineralization and improve the remineralization process including fluoride compounds, CPP-ACP and laser irradiation.

The present study aimed at evaluating the effect of the combined use of Er,Cr:YSGG hard tissue laser irradiation and CPP-ACP topical application on the prevention of demineralization of enamel. To our knowledge, no previous studies were done to evaluate their combined use on the prevention of enamel demineralization of permanent teeth. Therefore, a controlled in vitro study design using human premolar teeth was selected in order to be able to section the teeth and view them microscopically to detect the morphological alteration caused by their combined use.

Randomization and allocation was done using a randomization computer generated program to avoid examiner bias. Sample size was selected to include a minimum of 15 teeth per group calculated using a computer software using similar previous studies. Thus it was decided to include 20 teeth per group with a total of 80 teeth in order to compensate for any dropouts during the research.

Simulation of the oral environment was done by three aspects. First, human teeth were used and stored in an artificial saliva solution at neutral pH immediately after their extraction and throughout the research period to simulate the salivary composition intra-orally. Featherstone and Mellberg showed that bovine teeth enamel dissolves two or three times quicker than human enamel, that is why human premolar teeth were used in this study. Also, teeth were subjected to thermo cycling and brushing cycles equivalent to one year in the oral environment to mimic the in vivo conditions.

The control group showed the most severe WSLs formation as well as the highest lesions depth (328.56 µm) upon comparison with all other 3 groups which received different preventive measures. This emphasizes the beneficial effect of applying different preventive measures to reduce the incidence of WSLs occurrence.

The use of CPP-ACP in the current study was based upon the positive results established in previous studies that investigated the effect of different CPP-ACP formulations on the prevention of WSLs. In the present study, CPP-ACP was used in form of a tooth mousse (GC tooth mousse), applied according to the manufactures instructions. This has resulted in a significant reduction in the severity of WSLs formation and in 48.7% less lesion depth when compared to the control group. The results of the conducted study came in accordance with the results of different previous studies using different CPP-ACP formulations.

Rahiotis et al. tested the anti-cariogenic potential of CPP-ACP tooth mousse and found significantly lower demineralization when comparing it to the control group by surface analysis using Fourier transformance micro multiple internal reflectance infrared spectroscopy (micro MIR-FTIR). Scanning electron microscope examination of the enamel morphology performed by Oshiro et al. on bovine teeth showed the capability of CPP-ACP to prevent demineralization regardless of the use of dissimilar timing protocols for the demineralization-remineralization cycles to induce artificial WSLs. Moreover, a meta-analysis presented in 2009 concluded that the in vivo randomized control trials suggest caries preventive effect for CPP-ACP long-standing clinical usage.

Another preventive measure has been applied and tested in this study which is hard tissue laser irradiation using Er,Cr:YSGG laser. For caries prevention, laser irradiation should not ablate the surface, but change and modify...
the surface morphological, chemical composition or solubility of enamel instead, unlike what is used during cutting the enamel surface for caries removal.\(^{(48)}\) It was noted previously that enamel’s acid resistance relies on the laser energy applied, the higher the fluence, the greater the acid resistance. This fact is associated with the surface temperature reached.\(^{(49)}\) Accordingly, the Er,Cr:YSGG laser has a privilege over the Er:YAG laser in this perspective owing to its higher absorption to a peak of hydroxyapatite, so it appears more effective for caries prevention and that is why it was used in the present study.\(^{(50)}\) Sub-ablative parameters were used based on earlier studies\(^{(25,37,38)}\) since until now there is no specific protocol for laser irradiation for caries prevention. In the current study, water spray was not used during Laser application. Hossain and colleagues\(^{(51)}\) found that depths of the lesions formed after Er,Cr:YSGG irradiation with water spray were noticeably greater than those lacking water spray both in enamel and dentin. The authors reported that water plays a significant part in the ablation of tissues. In addition, Apel et al.\(^{(49)}\) reported that Er:YAG laser without water coolant was more effective in caries prevention than when it was used with water coolant.

In the current study, the application of Er,Cr:YSGG laser irradiation at sub-ablative parameters resulted in significantly less severe WSLs and 45% less lesion depth when compared to the control group. This percentage is comparable to the conclusion of an article in 2006\(^{(22)}\) that reviewed the literature regarding the laser effects on caries inhibition which revealed that the percentage inhibition of caries after laser irradiation ranged from 30% to 97.2% depending on the type of laser used, laser parameters and various confounding factors. Anaraki et al.\(^{(25)}\) invitro study results came in agreement to the presented results where they evaluated the Er,Cr:YSGG laser efficacy either assisted or unassisted with fluoride on enamel acid resistance using atomic absorption spectrometry. They reported that laser either by itself or assisted by fluoride produced significant reduction in enamel demineralization. In addition, Similarly, Hossain et al.\(^{(27)}\) found a significant caries preventive role of the Er,Cr:YSGG laser irradiation at 6W and 5W pulse energies, upon spectrophotometry and scanning electron microscope evaluation.

Although statistically insignificant, the laser group showed more enamel demineralization when being compared to the CPP-ACP group. Apel et al.\(^{(52)}\) noted that laser irradiation even at sub-ablative parameters (8J/cm\(^2\)), can yield fine cracks in enamel which might act as starting points for acid attack and this could explain why the laser group in the conducted study showed more demineralization than the CPP-ACP group.

The present study aimed to evaluate if the combination of laser irradiation with CPP-ACP has a significant synergistic effect on the prevention of enamel demineralization. The results of the present study supported the hypothesis that the combined use of Er,Cr:YSGG laser irradiation and CPP-ACP resulted in the least severe lesion formation after induction of artificial WSLs, as well as the least lesion depth of all groups (24.5 µm).

Different mechanisms could explain the improved resistance of enamel to demineralization observed when using laser irradiation in conjunction with the CPP-ACP. Firstly, recrystallization of the hydroxyapatite crystals, where formation of new crystallographic phases on enamel corresponding to \(\alpha\)-tricalcium phosphate, \(\beta\)-tricalcium phosphate and tetracalcium phosphate, larger than the initial ones were observed by X-ray diffraction in a study by Zezell et al.\(^{(53)}\) when evaluating enamel changes after irradiation by Er,Cr:YSGG and Nd:YAG lasers. These new crystal phases, in the presence of CPP-ACP, enhance the uptake and deposition of the calcium and phosphate ions in the enamel subsurface. This results in the localization of amorphous calcium fluoride phosphate at the tooth surface which co-localizes calcium, phosphate and fluoride from the saliva as bioavailable ions to produce fluoroapatite.\(^{(54)}\) This was also confirmed by Westerman et al.\(^{(55)}\) who observed an increase in calcium, phosphate and fluoride uptake from exogenous sources after laser irradiation.
Additionally, recrystallizing of enamel crystals made by laser irradiation results in better retention of calcium and phosphate ions when compared to the non-irradiated enamel, possibly due to entrapment and better penetration of these ions deep into the micro-spaces between granules produced by the recrystallization following laser irradiation besides the heat generated in depth by laser.\(^{(54)}\) Similarly, Zezell et al.\(^{(53)}\) concluded that the laser induced alterations on enamel might be the cause for increasing the formation and retention of CaF\(_2\)-like material detected, which might explain the reduction of enamel demineralization.

Moreover, the synergistic effect observed in the laser and CPP-ACP combination could be related to the purification of enamel hydroxyapatite. Westerman et al.\(^{(56)}\) found increased surface roughness and cracks in the enamel after laser irradiation when compared to other groups under a scanning electron microscope. Likewise, Subramanian and Pandey\(^{(57)}\) noted marked surface roughness after Er,Cr:YSGG laser irradiation. This comes in accordance to the conducted study, where surface roughness was observed in the laser alone group under the stereomicroscopic examination (Figure 9). Asl-Aminabadi et al.\(^{(54)}\) observed fine irregularities and cracks in the enamel surface in the laser alone group under the scanning electron microscope favoring brittleness of the enamel and causing a decrease in surface micro hardness in that group. However, these cracks were not seen in the CPP-ACP group and the CPP-ACP plus laser group. It was concluded that when laser was combined with CPP-ACP, the fluoride ions contacted the free calcium and phosphate ions forming new hydroxyapatite and fluoroapatite crystals, which coated the enamel cracks and irregularities produced by the laser to form a smoother and more homogenous glazed enamel surface resulting in the purification of enamel.\(^{(54)}\) Also, Niazy and Ehab\(^{(58)}\) stated that CPP-ACP application after laser irradiation allowed for the incorporation of nanocomplexes on the tooth surface micro-cracks. This finding was formerly noticed by Hicks and co-workers\(^{(21)}\), the authors reported that with argon laser irradiation before acidulated phosphate fluoride application, a homogenous confluent enamel surface was existent that masked typical enamel surface markings that were apparent in the argon laser alone group. Similarly, this was obvious in the current study in the stereomicroscopic examination of group IV (Figure 10).

**CONCLUSIONS**

Based on the results of the current study, it can be concluded that:

1. The combined use of Er,Cr:YSGG laser irradiation and CPP-ACP showed the best preventive measure against enamel deminerlization evidenced by the least severe WSLs and least lesions depth when compared to the use of laser alone, CPP-ACP alone and the control groups.

2. The use of CPP-ACP alone or Laser alone resulted also in a significant reduction in WSLs severity and depth when compared to the control group, however it was significantly less than their combined use.

3. No significant difference was recorded between the CPP-ACP alone or the laser group alone in reduction of enamel lesions severity and depth.

**REFERENCES**


