

Efficacy of erbium-doped yttrium aluminum garnet laser with casein phosphopeptide amorphous calcium phosphate with and without fluoride for remineralization of white spot lesions around orthodontic brackets

Sogra Yassaei¹, Hossein Aghili¹, Neda Shahraki², Isa Safari¹

Correspondence: Dr. Isa Safari
E-mail: isasafari1365@gmail.com

¹Department of Orthodontics, Faculty of Dentistry, Shahid Sadoughi University of Medical Sciences, Yazd, Iran,

²Department of Orthodontics, Faculty of Dentistry, Zahedan University of Medical Sciences, Zahedan, Iran

ABSTRACT

Objective: This study aimed to assess the efficacy of erbium-doped yttrium aluminum garnet (Er:YAG) laser, pastes containing casein phosphopeptide amorphous calcium phosphate (CPP-ACP) with and without fluoride and their combination for prevention of white spot lesions in the enamel. **Materials and Methods:** This *in vitro* experimental study was conducted on 90 extracted sound premolars. The teeth were then randomly divided into six groups of 15: (1) Control, (2) laser, (3) CPP-ACP with fluoride (GC MI Paste, Recaldent™ 900 ppm as NaF), (4) CPP-ACP without fluoride (GC Tooth Mousse Recaldent™), (5) laser + CPP-ACP with fluoride, and (6) laser + CPP-ACP without fluoride. In each group, enamel surface was exposed to the remineralizing agent. The teeth were then subjected to pH cycling for 14 days. The teeth were then sectioned, polished, and underwent cross-sectional microhardness testing at 20–160 μ depth quantitatively. Using the Simpson's rule, the amount of mineral loss was calculated in each group. Statistical Analysis Used: ANOVA was used for the comparisons, and Tukey's test was applied for pairwise comparisons. **Results:** The highest mean volume percentage of microhardness at 20–60 μ depth belonged to the group laser + CPP-ACP with fluoride and the lowest belonged to the control group ($P = 0.001$). The differences were not significant at 80–120 μ depth ($P > 0.05$). These findings are confirmed according to ΔZ (mineral loss). **Conclusion:** Based on these results, Er: YAG laser was able to decrease demineralization and was a potential alternative to preventive dentistry and was more effective when combined with CPP-ACP products.

Key words: Casein phosphopeptide amorphous calcium phosphate, erbium-doped yttrium aluminum garnet laser, white spot lesion

INTRODUCTION

Enamel demineralization and formation of white spot lesions (WSLs) around orthodontic brackets are

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a common problem, especially in patients with poor oral hygiene.^[1,2] The prevalence of WSLs is 50%–95% in patients using fixed orthodontic appliances.^[3,4]

Early detection and treatment of WSLs during orthodontic treatment is highly important because these lesions are reversible. Casein phosphopeptide amorphous calcium phosphate (CPP-ACP) products were recently introduced for caries prevention and remineralization of demineralized enamel.^[5,6] CPP-ACP is applied on the tooth surface and dental plaque and when exposed to acid, it releases calcium and phosphate ions. Subsequently, the concentration of calcium and phosphate ions increases in the oral cavity and thus, they deposit on the tooth surface, prevent caries, and enhance remineralization.^[7] Evidence shows that CPP-ACP compounds can decrease demineralization and increase remineralization of enamel *in vivo* and *in vitro*.^[8-10] Iijima *et al.*^[11] in their clinical study showed that the addition of 18.8 mg CPP-ACP to sugar-free gums increased the enamel resistance to demineralization and enhanced remineralization. CPP-ACP is also added to some fluoride compounds, and it has been claimed that these products have higher potential for enamel remineralization due to fluoride release.^[12-14] Guotoa *et al.*^[15] assessed the efficacy of tooth mousse (GC, Tokyo, Japan)-containing CPP-ACP for enamel remineralization using circulatory polarized image and concluded that it can decrease the size of demineralized lesions and increase enamel remineralization; its combination with fluoride further increases its remineralizing efficacy.

Recently, laser irradiation was suggested for caries prevention. CO₂, erbium, and erbium-doped yttrium aluminum garnet (Er:YAG) lasers are effective for caries prevention.^[16-21] These lasers are highly absorbed by water and hydroxyapatite crystals and can change their crystalline structure and increase their resistance to demineralization.^[19]

Jeng feng liu *et al.*^[22] evaluated the efficacy of Er:YAG laser for caries prevention and concluded that this laser can be an efficient tool for the prevention of enamel demineralization. Efficacy of remineralizing agents and laser has been previously evaluated but studies on their synergistic effect on caries prevention are limited. Thus, this study aimed to assess the efficacy of Er:YAG laser irradiation along with (CPP-ACP) with and without fluoride for remineralization of WSLs *in vitro*.

MATERIALS AND METHODS

This *in vitro* study was conducted on 90 premolar teeth extracted for orthodontic reasons. The teeth were rinsed with saline and immersed in 0.1% thymol solution. Premolar teeth with abnormal anatomy, cracks, or caries were excluded from the study. The teeth were cleaned with nonfluoridated pumice paste and rubber cup and dried for 15 s.

To prevent demineralization, the entire tooth surface, except for a rectangular-shaped window measuring 5 mm × 6 mm, was coated with acid-resistant nail varnish (Revlon, New York, NY, USA). The teeth were then randomly divided into six groups (*n* = 15) as follows:

- G1 or control group: The teeth underwent pH cycling with no extra intervention
- pH-cycling process: The teeth were immersed in a demineralizing agent (2.0 mmol/L calcium, 2.0 mmol/L phosphate, and 0.03 mg fluoride in 75 mmol/L acetate buffer with a pH of 4.3) manufactured in School of Chemistry of Yazd University for 3 h daily and were then immersed in a remineralizing agent (1.5 mmol/L calcium, 0.9 mmol/L phosphate, and 150 mmol/L KCL in 20 mmol/L cacodylate buffer with a pH of 7.4) for 20 h. This process was continued for 10 days, and then, the teeth were immersed in the remineralizing agent at 37°C for two more days. During the time intervals between immersions and after completion of pH cycling, the teeth were rinsed with saline for 10 s and dried with paper towel. Both solutions were refreshed after every five cycles, and 1% thymol was added to prevent the growth of microorganisms
- G2 or Er: YAG laser: This group was only subjected to laser radiation
- G3 or CPP-ACP with fluoride (GC MI Paste Plus, Recaldent™ 900 ppm as NaF, GC Corporation Tokyo, Japan): In this group, after drying the enamel, the respective area was exposed to MI Paste Plus with 900 ppm fluoride (CPP-ACP technology, School of Dental Science, Melbourne, Australia) and rinsed with saline after 5 min
- G4 or CPP-ACP without fluoride (GC Tooth Mousse, Recaldent™ GC Corporation Tokyo, Japan): After drying the enamel, the respective area was exposed to GC Tooth Mousse (CPP-ACP technology, School of Dental Science, Melbourne, Australia) and rinsed with saline after 5 min
- G5 or Er: YAG laser + CPP-ACP with fluoride: In this group, the respective area was laser irradiated and then MI Paste Plus was applied

- G6 or ER:YAG laser + CPP-ACP without fluoride: In this group, the respective site was laser irradiated and then GC Tooth Mousse was applied.

In the laser groups (G2, G5, and G6), the teeth were subjected to Er:YAG laser (KEY Laser 3+, Kavo Dental Corp., Biberach, Germany) irradiation with 2.94 μm wavelength, 80 mJ energy, 4 Hz frequency and 80 mJ energy per pulse for 10 s under water and air spray with 2060 hand piece (Kavo Dental Corp., Biberach, Germany) at 20 mm distance from the surface in nonfocal mode and perpendicular to the tooth surface with a swiping motion. Next, the teeth in all groups were subjected to pH cycling to artificially create WSLs. Next, the teeth were sectioned at the middle of the exposed enamel surface by a cutting saw and each section was mounted in polyester resin.

Considering the need for polishing of the surface before microhardness testing, the slices were first polished by Soflex polishing discs (3M ESPE, St. Paul, MN, USA) serially from coarse to fine under water coolant and then subjected to further polishing with silicon carbide rotary discs starting from 6000 grit to the finest grit under water coolant.

The hardness profile was then measured using a hardness tester (FM-700 type D, Future-Tech, Kawasaki, Japan) by a Knoop diamond indenter with 10 g load applied for 5 s at 20, 40, 60, 80, 100, 120, 140, and 160 μm distances from the external border of the enamel. To increase accuracy, hardness was measured at three points at each depth, and then, the Knoop hardness number (KHN) was converted to volume percentage of mineral content using the formula below:^[23]

$$\text{Vol\% mineral} = 4.3 (\text{KHN})^{1/2} + 11.3$$

To obtain the relative mineral loss (ΔZ), the volume percentage values were graphed against distance and using Simpson's rule for calculation of the integral of area under the curve, the integral of mineral loss for

each group was calculated such that first, the integral of sound enamel and then the integral of affected enamel were calculated and subtracted to obtain the amount of mineral loss.

Data were analyzed using PASW version 18 (SPSS, Chicago, IL). ANOVA was used for the comparisons, and Tukey's test was applied for pairwise comparisons.

RESULTS

ANOVA found a significant difference in the mean hardness value of different groups at 20–60 μ depths [$P < 0.0001$, Table 1].

According to Table 1, the mean volume percentage of hardness at 20 μ depth in different groups was in the range of 54.85–94.79. At 20 μ depth, the highest volume percentage of hardness belonged to the laser + CPP-ACP with fluoride group (83.53 ± 5.59) while the lowest was noted in the control group (62.78 ± 5.23) ($P = 0.001$). The same trend was observed at 40 and 60 μ depths. ANOVA showed a significant difference in the mean hardness value of the groups at 20–60 μ depths ($P = 0.001$). The differences were not significant at 80–120 μ depth ($P > 0.05$) [Table 1 and Figure 1].

Table 2 shows the mean relative mineral loss (ΔZ) in different groups. The highest mineral loss was noted in the control group (1902.26 ± 141.38) while the lowest belonged to CPP-ACP with F + laser group (900.49 ± 164.29). ANOVA test showed a significant difference between relative mineral loss (ΔZ) in six groups ($P = 0.001$).

Table 3 shows the results of Tukey's test. According to Table 3, laser and CPP-ACP without fluoride + laser groups were not significantly different in terms of ΔZ . In addition, laser and CPP-ACP with fluoride groups were similar ($P > 0.05$). CPP-ACP

Table 1: Mean volume percentage of hardness in different groups at different depths

Groups	Mean \pm SD							
	20 depth	40 depth	60 depth	80 depth	100 depth	120 depth	140 depth	160 depth
CPP-ACP with fluoride	72.20 \pm 7.33	74.97 \pm 6.67	78.11 \pm 4.26	91.44 \pm 3.29	89.86 \pm 5.73	92.41 \pm 3.88	91.88 \pm 2.62	93.80 \pm 3.80
Laser	75.51 \pm 4.44	78.78 \pm 3.56	79.70 \pm 4.31	89.58 \pm 4.35	91.06 \pm 3.10	90.80 \pm 3.06	92.42 \pm 3.52	92.36 \pm 4.26
CPP-ACP without fluoride	70.47 \pm 4.37	72.20 \pm 4.65	77.14 \pm 4.27	89.72 \pm 4.63	90.19 \pm 3.75	90.70 \pm 3.35	91.29 \pm 4.30	92.56 \pm 3.96
CPP-ACP without fluoride + laser	79.57 \pm 5.65	80.14 \pm 3.11	80.45 \pm 5.41	89.01 \pm 5.55	91.86 \pm 4.58	92.31 \pm 4.06	91.50 \pm 4.38	91.99 \pm 4.59
CPP-ACP with fluoride + laser	83.53 \pm 5.59	83.50 \pm 6.47	82.93 \pm 8.92	91.49 \pm 3.60	91.45 \pm 3.57	91.82 \pm 4.22	91.43 \pm 3.19	92.32 \pm 3.97
Control	62.78 \pm 5.23	61.61 \pm 3.86	63.25 \pm 6.31	88.90 \pm 7.75	91.46 \pm 4.16	90.91 \pm 4.15	92.14 \pm 4.15	91.81 \pm 4.93
Significant	0.0001*	0.0001*	0.0001*	0.597	0.762	0.676	0.958	0.841

The mean difference is significant at the 0.05 level. CPP-ACP: Casein phosphopeptide amorphous calcium phosphate, SD: Standard deviation

Table 2: Mean mineral loss (ΔZ) values of the groups

Groups	n	Mean \pm SD
Control	15	1902.26 \pm 141.38
CPP-ACP without fluoride	15	1377.87 \pm 155.97
CPP-ACP with fluoride	15	1268.14 \pm 176.24
Laser	15	1135.64 \pm 109.93
CPP-ACP without fluoride + laser	15	1066.11 \pm 134.02
CPP-ACP with fluoride + laser	15	900.49 \pm 164.29
Significant		0.000

The mean difference is significant at the 0.05 level.
CPP-ACP: Casein phosphopeptide amorphous calcium phosphate

with F + laser significantly different the other groups ($P < 0.05$).

DISCUSSION

Despite advances in orthodontic materials and techniques, enamel demineralization around orthodontic appliances known as WSL remains a serious clinical problem, especially in patients with poor oral hygiene. WSLs develop due to long-term accumulation of bacterial plaque, which causes acidic dissolution of enamel.^[15]

Evidence shows that the prevalence of decalcification in orthodontic patients varies from 50 to 95%.^[34] O'Reilly and Featherstone^[24] showed that demineralization around orthodontic brackets may occur within 1 month in patients who use fluoridated toothpaste twice a day. High incidence and fast initiation of WSLs necessitate the use of preventive measures.

Aside from visual examination, which is the most commonly used technique in clinical examination for detection of WSLs, other methods are available for quantification and comparison of enamel demineralization including microhardness measurement, microradiography, polarized light microscopy, QLF, confocal microscopy, and laser fluorescence.^[25] Since enamel hardness is affected by its mineral content, microhardness test is extensively performed *in vitro* to assess enamel demineralization. The previous studies showed that microhardness test is a reliable, reproducible, and quantitative method for the assessment of demineralization.^[26] Of methods used for measurement of microhardness, the Knoop test has the lowest rate of measurement errors (<5%). Moreover, evidence shows that a linear correlation exists between the enamel KHN and mineral content of the enamel^[27] such that correlation coefficient of 91% has been reported between hardness test and volume percentage of minerals.^[26] Thus, Knoop microindentation test was performed

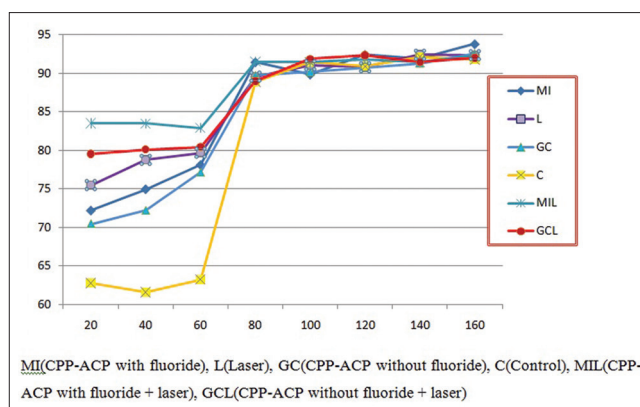


Figure 1: Comparison of volume of different groups at different depths

in our study for quantitative measurement of enamel demineralization.

To determine the hardness profile, hardness must be measured at specific distances from the external enamel surface. In previous studies, indentation has been performed at different distances from the surface.^[27-30] In the current study, the first indentation was made at 20 μ distance from the surface and continued to 160 μ depth. The reason behind selection of these distances was prevention of surface cracks, which occur if the indentation is made close to the surface and compromise accurate measurement. In addition, by keeping 10 μ distances, risk of super positioning of indenter exists while greater distances complicate the detection of small changes of hardness at different depths of the enamel.^[27]

In the current study, the mean hardness, volume percentage of mineral content, and relative mineral loss (ΔZ) in different groups were analyzed using one-way ANOVA and *post hoc* Tukey's test.

CPP-ACP is a natural derivative of milk and due to its high solubility, it can hydrolyze into apatite in the oral environment, making it a primary candidate for remineralization therapy.^[29]

Evidence shows that CPP-ACP products prevent enamel demineralization and enhance remineralization *in vitro* and *in vivo*.^[31]

CPP-ACP has been proved to have anti cariogenic activity in human studies^[9,32] although some studies suggest that the addition of CPP-ACP has no significant advantage over fluoride toothpaste.^[33,34] The evidence for the effectiveness of CPP-ACPF applications on the reduction of DWLs once orthodontic appliances have been removed is equivocal. Two studies found a

Table 3: Statistical comparison of relative mineral loss of enamel lesions in groups (Tukey test)

Group (A)	Group (B)	Mean difference (A-B)	Significant
Control	CPP-ACP without fluoride	524.39*	0.000
	CPP-ACP with fluoride	634.12*	0.000
	Laser	766.62*	0.000
	CPP-ACP without fluoride + laser	836.15*	0.000
	CPP-ACP with fluoride + laser	1001.76*	0.000
CPP-ACP without fluoride	Control	-524.39*	0.000
	CPP-ACP with fluoride	109.73	0.339
	Laser	242.23*	0.001
	CPP-ACP without fluoride + laser	311.76*	0.000
	CPP-ACP with fluoride + laser	477.38*	0.000
CPP-ACP with fluoride	Control	-634.12*	0.000
	CPP-ACP without fluoride	-109.73	0.339
	Laser	132.49	0.154
	CPP-ACP without fluoride + laser	202.03*	0.001
	CPP-ACP with fluoride + laser	367.64*	0.000
Laser	Control	-766.62*	0.000
	CPP-ACP without fluoride	-242.23*	0.001
	CPP-ACP with fluoride	-132.64	0.154
	CPP-ACP without fluoride + laser	69.53	0.794
	CPP-ACP with fluoride + laser	235.14*	0.001
CPP-ACP without fluoride + laser	Control	-836.15*	0.000
	CPP-ACP without fluoride	-311.76*	0.000
	CPP-ACP with fluoride	-202.03*	0.001
	Laser	-69.53	0.794
	CPP-ACP with fluoride + laser	165.62*	0.001
CPP-ACP with fluoride + laser	Control	-1001.76*	0.000
	CPP-ACP without fluoride	-477.38*	0.000
	CPP-ACP with fluoride	-367.64*	0.000
	Laser	-235.14*	0.001
	CPP-ACP without fluoride + laser	-165.62*	0.001

*The mean difference is significant at the 0.05 level.

CPP-ACP: Casein phosphopeptide amorphous calcium phosphate

positive effect of using a CPP-ACP containing cream, in addition to fluoride,^[35,36] and three studies found a limited effect;^[38-40] therefore, further work in this area is required before a definitive answer is found. The difference between our results and the mentioned studies could be due to fact that, our study was a laboratory study while, those studies were performed *in vivo*.

In the current study, mineral loss in CPP-ACP with fluoride and without fluoride groups was significantly less than that in the control group ($P < 0.0001$). This indicates the positive efficacy of these materials for prevention of enamel demineralization. In addition, demineralization in CPP-ACP with fluoride group containing 900 ppm fluoride was less than that in the CPP-ACP without fluoride group; although this difference was not statistically significant. These results were in agreement with those of Wu *et al.*^[15] Iijima *et al.*^[11] and Elsayad *et al.*^[41] which showed the greater efficacy of combination of CPP-ACP and fluoride for the prevention of enamel demineralization.

Kumar *et al.*^[41] compared the effect of CPP-ACP with fluoridated and nonfluoridated toothpastes and concluded that combination of CPP-ACP and fluoride was more effective than toothpastes for remineralization of WSLs. Their results were in agreement with ours regarding the optimal efficacy of CPP-ACP with fluoride (MI Paste Plus). Jayarajan *et al.*^[14] compared the efficacy of MI Paste Plus, CPP-ACP, and artificial saliva and concluded that MI Paste Plus was more effective than other groups due to the presence of fluoride in its composition; their study was somehow similar to our study regarding the materials tested and the results obtained. In our study, remineralization in CPP-ACP with fluoride and without fluoride groups was higher than that in the control group, and CPP-ACP with fluoride group showed slightly higher remineralization than CPP-ACP without fluoride group such that mineral loss at the first three depths was 68% in the control group, 36% in CPP-ACP without fluoride group and 32% in CPP-ACP with fluoride group. One advantage of CPP-ACP compared to fluoride is that CPP-ACP does not cause fluorosis. Thus, application of CPP-ACP alone or in combination with fluoride may decrease the amount of fluoride and lower the risk of fluorosis.^[42]

Use of lasers for caries prevention has been an interesting topic of research in the recent years. Laser irradiation of teeth causes reactions between laser light and biologic materials in tooth structure. If laser light is absorbed by the enamel, its energy is converted to heat; these thermal effects cause chemical and microstructural changes in the enamel and increase its resistance to acid attacks. In demineralization tests, heating the enamel to 300°–400°C decreases its relative solubility and the depth of lesions. Several theories have tried to explain the reduction in acidic dissolution of enamel following heating. In addition to decreased

permeability of enamel following laser irradiation, release of bonded carbonate following enamel heating is a more acceptable theory. A direct correlation exists between carbonate loss following laser irradiation and decreased acidic dissolution of enamel. Moreover, the amount of hydroxide and pyrophosphate also increases following laser therapy.^[43]

The ability of different wavelengths of erbium laser (erbium:yttrium-scandium-gallium-garnet, Er:YAG) for increasing the enamel resistance to acids has been previously evaluated

Liu *et al.*^[22] de Andrade *et al.*^[44] and Apel *et al.*^[45] evaluated the effect of Er:YAG laser on enamel resistance to caries and indicated increased resistance of enamel to acids following subablative laser irradiation, which was in accord with our results. However, Apel *et al.*^[45] stated that prevention of unwanted ablation of enamel is difficult and cannot be achieved with the currently available lasers and wavelengths.

Nair *et al.*^[46] compared the efficacy of remineralizing agents in combination with Er:YAG laser for prevention of caries and reported the highest release of calcium ions in CPP-ACP group combined with laser. Their results were in line with ours. They used spectrometry for the assessment of demineralization while we used microhardness test as a standard and reproducible technique with less than 5% error rate. Yassaei *et al.*^[47] compared the efficacy of combined effects of Er:YAG laser and MI Paste plus on the inhibition of enamel demineralization. In the aforementioned three studies, laser irradiation combined with topical application of remineralizing agents had a greater efficacy for prevention of enamel demineralization, which was in agreement with the results of the current study showing the least demineralization in CPP-ACP with fluoride + laser and CPP-ACP without fluoride + laser groups.

CONCLUSION

Based on these results, Er:YAG laser was able to decrease demineralization and was a potential alternative to preventive dentistry and was more effective when combined with CPP-ACP products

Suggestions

In vitro studies have limitations in simulation of oral environment. Thus, the effect of these products on WSLs should also be evaluated in clinical studies.

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Conflicts of interest

There are no conflicts of interest.

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