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Original Article

Combined effects of Er: YAG laser and casein phosphopeptide-amorphous calcium phosphate on the inhibition of enamel demineralization: An *in vitro* study

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ABSTRACT

Background: Development of white spot lesions on enamel is a significant and common problem during the fixed orthodontic treatment. Various preventive methods have been suggested. The purpose of this study was to evaluate the preventive potential of MI Paste Plus, Er:YAG Laser and combined under similar *in vitro* conditions against demineralization.
Materials and Methods: In this experimental *in vitro* study, 60 extracted premolars were randomly allocated to four groups (n = 15) of control, MI Paste Plus, Laser and MI + Laser (MIL). Enamel surface of each group was treated with one of above materials before and during the pH cycling for 12 days through a daily procedure of demineralization and remineralization for 3 h and 20 h, respectively. Teeth were sectioned and evaluated quantitatively by cross-sectional microhardness testing at 20 μm intervals from the outer enamel surface toward dentinoenamel junction up to 160 μm and data were analyzed using the one-way analysis of variance and Tukey test. $P < 0.05$ was considered as significant.
Results: MIL group had the least amount of demineralization ($P < 0.001$). Control group (C group) had the greatest relative mineral loss and the laser group (L group) had 45% less mineral loss than the C group and there was no significant difference between the MI Paste Plus and L group ($P = 0.154$)
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 casein phosphopeptide-amorphous calcium phosphate products.

Key Words: Er: YAG, , laser, MI Paste Plus, white spot lesion

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INTRODUCTION

Demineralization of enamel and subsequently creation of white spot lesions (WSLs) around orthodontic brackets is a common problem in fixed orthodontics, particularly with poor oral hygiene.[1,2] Gorelick *et al*. reported prevalence of about 50% of fixed orthodontic cases with WSL;[3] although, recent studies reported 73-95%.[4,5] Demineralized lesions in significant depth (75-μ m) can develop in 1 month or less when orthodontic appointment intervals are set at 6-10 weeks.[3,6] Early detection of WSLs during orthodontic treatment is of great importance because these lesions are unworthy, unhealthy and potentially irreversible.[3] Therefore, various products and preventive measures are introduced to reduce the severity of this problem.

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In recent years, calcium phosphate-based remineralization technologies have been demonstrated as adjunct to fluoride therapy in non-invasive treatment of WSL.[7,8] Iijima *et al*. reported that casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) decreased demineralization, increased remineralization and created remineralized enamel that was more resistant to future demineralization.[9] Other studies have shown similar results.[10-13] High solubility, ability for rapid hydrolyzation to form apatite molecule in the oral environment and the fact that CPP-ACP does not have adverse effects on taste have made it the 1st candidate for remineralization therapy.[14] However, other studies have shown no clinical advantage for the use of CPP- amorphous calcium fluoride phosphate paste supplementary to normal oral hygiene.

Since the first report of Stern and Sognnaes[15] about laser in1966, it was suggested that laser irradiation could increase the acid resistance of enamel. The lasers which are highly absorbable by dental enamel such as CO2 (9.6 μ m and 10.6 μ m), Er:YAG (2.94 μ m) and Er, Cr:YSGG (2.79 μ m) were employed with success.[16] Some authors[17] reported that Er:YAG laser could be used as a preventive tool against enamel demineralization. High energy per pulse and more pulses per second could result in a more resistant enamel surface to caries formation.[17]

To prevent from dental caries, laser irradiation should create chemical changes in dental enamel to decrease its solubility. There are some theories about the mechanism of increasing acid resistance of enamel following irradiation: (1) decreasing the permeability of enamel through melting and recrystallizing of enamel surface; (2) decreasing the solubility of enamel through the formation of less soluble particles like tetracalcium diphosphate monoxide; (3) reducing the water and carbonate contents, increasing the hydroxyl ion content and formation of pyrophosphate. The crystallographic changes of enamel are promoted by heating the surface by radiation.[16]

Although the effect of laser irradiation on acid resistance of enamel is recognized, the benefit of combined application of laser irradiation and CPP-ACP products is not clearly established. Thus, the aim of the present study was to investigate the preventive potential of Er:YAG laser and CPP-ACP products against demineralization of enamel, *in vitro*.

MATERIALS AND METHODS

Tooth selection and sample preparation

In this experimental *in vitro* study, 60 extracted caries-free human premolars were collected and washed with normal saline and stored in 0.1% thymol solution. Then, each tooth was cleaned with non-fluoridated pumice by a rubber cup on slow speed hand piece and residual material was washed away with normal saline then air-dried for 15 s.

To avoid the interference from demineralizing the entire crown, teeth had all surfaces covered with acid resistant nail varnish except for a window (4.0 mm × 5.0 mm) in the middle-third of buccal surfaces.

Then, the teeth were divided randomly into four groups of 15.

Group 1: control group (C group), the teeth were only introduced to the pH cycling regimen without any treatment.

Group 2: MI Paste Plus group (M group), teeth in this group were dried and the exposed enamel in the window was covered with a pie size amount of MI Paste Plus (GC America Inc.

Alsip, IL, USA) by cotton swab. It was left undisturbed for 3 min. The MI Paste on each tooth was removed by a cotton roll, but allowed the remnants be sat for another 2 min. Finally, the remaining residue was washed away with normal saline. To follow the manufacturer's instruction, we applied the MI Paste Plus daily during the experiment (before and during the pH cycling).

Group 3: Laser group (L group).

Group 4: MI + Laser (MIL) group, in this group following laser irradiation; we applied MI Paste Plus similar to M group.

Laser irradiation

Before the pH cycling, an Er:YAG laser emitting at a wavelength of 2.94-mm was used to irradiate the exposed enamel. The parameters used were 80 mJ of energy and a 4 Hz frequency for 10 s and energy per pulse of 80 mJ with water spray and 1.2 mm spot size. The 2060 hand piece (Kavo Dental Corp., Biberach, Germany) with irradiation distances of 20 mm in a non-contact mode was used. The operation was performed by clamping the hand piece of the laser and moving the teeth manually.

pH cycling phase

The pH-cycling model used in this study was based on the one described by Featherstone *et al*.[18] and modified by Argenta *et al*.[19] Every day, the samples were individually immersed in a demineralizing solution containing 2.0 mmol/L calcium, 2.0 mmol/L phosphate in 75 mmol/L acetate buffer (pH 4.3) for 3 h and then 21 h in a remineralizing solution containing 1.5 mmol/L calcium, 0.9 mmol/Lphosphate and150 mmol/L KCl in 20 mmol/L cacodylate buffer (pH 7.4). Both of those solutions were made in the school of chemistry of the University of Yazd. This pH-cycling process was carried out for 12 days and after which, the teeth were kept in the remineralizing solution for 2 more days. Between immersing in demineralizing and remineralizing solutions and at the end of the pH cycling regime, the teeth were rinsed with normal saline for 10 s and wiped by tissue paper. The demineralizing and remineralizing solutions, both containing thymol to prevent microorganisms grow, were changed after each five cycles.

Microhardness profile

After completion of pH cycling, the crowns were thoroughly rinsed with normal saline and sectioned longitudinally through the exposed enamel. Half of each specimen was embedded in epoxy resin so that the cut surface was exposed. Samples were polished using wet 6000 grade grit laboratory silicon carbide grinding paper on rotary devices. After serial polishing, microhardness profile of each lesion was assessed at 20 μm intervals up to 160 μm across the cut surface by a microhardness tester (FM -700 type D, Future-Tech, Kawasaki, Japan) with a Knoop diamond indenter under a 10-g load for 5 s. The first indentation was made at the point 20 μm of depth from the outer enamel surface. Indentations were made across the sectioned lesion along in a line perpendicular to the surface toward the dentinoenamel junction (DEJ). Three rows of indentations were made and then the mean of diagonal length of the 3 indentations at each depth was calculated for each sample.

Then, Knoop hardness (KHN) values were converted into volume percent mineral (VPM) by using the equation proposed by Featherstone *et al*.[20]

For depths greater than 80 μm, a mean value of VPM was used as sound enamel. The average VPM of sound enamel is 90%. Therefore, values of VPM for depths between 80 μm and 160 μm from the outer surface were mathematically normalized to an average of 90% mineral so that each specimen could be directly compared with others. Mineral content of each group was used to calculate the relative mineral loss (ΔZ) by utilizing the Simpson's rule. The unit of ΔZ is defined by multiplying volume percent by distance.[21]

Statistical methods

Normality of the data distribution was tested by Kolmogrov-Smirnov test. We used analysis of variance (ANOVA) to determine whether there were any significant differences in the mean value of ΔZ between groups. A *post-hoc* Tukey comparison tests was performed to identify the statistically significant differences ($P < 0.05$) between the groups. The statistical analysis was performed by using IBM SPSS software (version 19.0.1).

RESULTS

The mean relative mineral loss (ΔZ), standard deviations, ranges, minimum and maximum measurements are summarized in Table 1. Group C had the greatest relative mineral loss and group MIL had the least. One-way ANOVA test showed a significant difference between ΔZ in four groups ($P < 0.000$). Pair-wise comparison by Tukey *post-hoc* test showed that group MIL had significantly less demineralization ($P < 0.001$) than the other groups (Table 2).

The enamel mineral content profile for the lesion areas in each group are shown in Figure 1. The mineral content at 20 μm depth in the group C was 63% and showed approximately 27% mineral loss. Compared with the mineral content of sound enamel

Table 1: Mean relative mineral loss (ΔZ) of enamel lesions in different groups

Group	*n*	Mean ΔZ*	SD	Min	Max
C group	15	1902.26	141.38	1696.41	2192.36
M group	15	1268.14	176.24	962.79	1723.2
MIL group	15	900.49	164.29	723.73	1184.17
L group	15	1135.64	109.93	941.37	1316.59

M: MI Paste Plus; C: Control; L: Laser; MIL: MI Paste Plus+laser; *Unit of mean ΔZ; volume %×μm

Table 2: Statistical comparison of relative mineral loss of enamel lesions in groups (Tukey test)

(I) group	(J) group	Mean difference (I-J)	Sig.	95% confidence interval	
				Lower bound	Upper bound
C	M	634.12*	0.000	475.85	792.39
	L	766.62*	0.000	608.35	924.89
	MIL	1001.76*	0.000	843.49	1160.03
M	C	−634.12*	0.000	−792.39	−475.85
	L	132.49	0.154	−25.76	290.76
	MIL	367.64*	0.000	209.37	525.91
L	M	−132.49	0.154	−290.76	25.76
	C	−766.62*	0.000	−924.89	−608.35
	MIL	235.14*	0.001	76.87	393.41
MIL	M	−367.64*	0.000	−525.91	−209.37
	L	−235.14*	0.001	−393.41	−76.87
	C	−1001.76*	0.000	−1160.03	−843.39

*The mean difference is significant at the 0.05 level; M: MI Paste Plus; C: Control; L: Laser; MIL: MI Paste Plus+laser

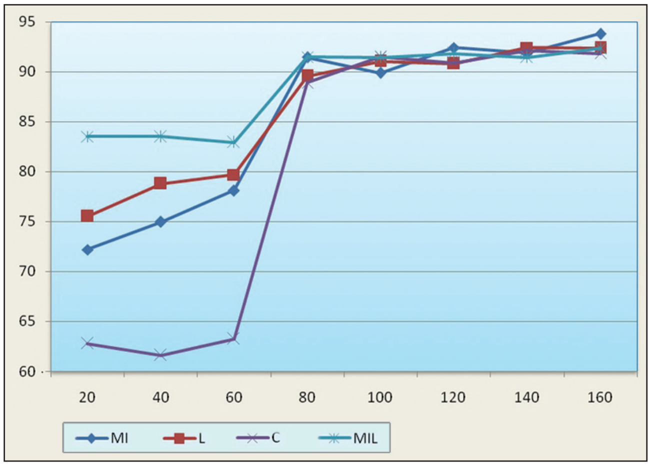

Figure 1: Comparative microhardness profile as volume percent mineral versus depth

(90%), it was 62% at 40 µm, 63.5% at 60 µm and 88% at 80 µm depths.

Teeth treated with MI Paste Plus showed 20% more mineral content than group C at 20 µm up to 60 µm depths, which indicates a partial inhibition of demineralization.

DISCUSSION

In this *in vitro* study, we examined the effect of MI Paste Plus, Er:YAG laser and combined on preventing enamel demineralization by the cross-sectional microhardness testing. Demineralization of enamel adjacent to orthodontic brackets is a widely studied topic and research about CPP-ACP and the laser is also a hot topic.[22] Methods to measure and compare enamel demineralization include visual examination, quantitative light-induced fluorescence, polarized light microscopy, microradiographs and microhardness testing. Since enamel hardness is thought to be related to its mineral content, the microhardness test is widely used in laboratory researches to study enamel demineralization.[23,24] Previous studies have shown that microhardness testing is a reliable, simple, quantitative and reproducible technique.[25] Among the methods to evaluate microhardness, the operator's error in KHNtest was found to be less than 5%.[26] In addition, a linear relationship between KHN and mineral content[25] and a correlation coefficient of 0.91 was found between the hardness and the volume percent of mineral content.[27] Therefore, in this study, Knoop microindentation was used as a quantitative technique to determine the amount of enamel demineralization. According to previous studies[19,28,29] an interval of 20 µm toward DEJ was kept between indentations on each row. Some studies have been making larger intervals of 25 µm[25,30] or as small as 10 µm.[26] A 20 µm interval between indentations was accomplished in order to avoid possible superimposition of indentations when working with a 10 µm interval. A larger interval could compromise the observation of small changes of hardness in different depths.[27]

CPP-ACP is recommended by the manufacturer as a home-care product and is claimed that remineralizes early enamel demineralization.[31] The anticariogenic effects of CPP-ACP is related to increasing the buffering effect of saliva leading to suppression of demineralization and activation of remineralization or both as a result of stabilization of calcium phosphate in solution through binding ACP to multiple phosphoserine residues and forming small clusters of CPP – ACP.[7] When CPP-ACP is combined with fluoride, the fluoride ions react with to form casein phosphopeptide-amorphous calcium fluoride phosphate.[31] In many studies, the remineralizing potential of CPP-ACP has been demonstrated; although, the methods of applying and assessment were different from ours.[32] Reynolds et al. established that MI Paste Plus, provided calcium, phosphate and fluoride ions for the surface of teeth and therefore had a tremendous effect on enamel remineralization,[13] the results of our study are similar to these studies because the MI Paste Plus (CPP-ACP + 900 ppm fluoride) group showed 32% mineral loss compared to group C (68%). The results from our study showed that the teeth treated with MI Paste Plus had less enamel demineralization than the control, which are in line with previous reports.[7-11]

In the last 36 years, many studies have shown the potential benefit of laser irradiation to dental tissues to inhibit enamel dissolution or formation and progression of artificial caries like lesions in laboratory.[33-35] Irradiation of teeth with laser light results in interaction between the light and the biological materials of dental hard substance. If the light is absorbed by the specific components, the irradiated energy converts directly into heat. This thermal effect is supposed to be the cause of microstructural and chemical changes occurring in dental enamel and explains the increased acid resistance of enamel surface. Heating dental enamel to 300-400°C leads to a relative reduced solubility of enamel, along with the shallowest lesions. There are various theories explaining the reduced acid solubility of dental enamel after heating. For instance, the water permeability of dental enamel is seen to be lower after heating. More hydroxide and pyrophosphate, but less carbonate, is also generally found in comparison with unheated enamel.[35] Er:YAG laser has been tested and has shown significant effects on increasing the acid resistance of dental enamel.[36]

A 23% mineral loss was found in our study using 80 mJ Er:YAG laser. This is similar to previous studies[37-39] showing a 5-67% reduction in enamel demineralization after irradiation with Er:YAG laser. A marked (40%) caries inhibition was achieved by Fried *et al*.[39] using sub-ablative laser parameters. Apel *et al*.[40] have also reported a significant decrease in enamel solubility after laser irradiation.

In this study, the mineral content of teeth in the MIL group at 20-60 μm depths were more than the other groups and showed that the effect of Er:YAG laser associated with CPP-ACP products on prevention of demineralization was more.

CONCLUSION

Within the limitations of our study and based on these results, Er:YAG laser was able to decrease the demineralization and can be a potential alternative to preventive dentistry and was more effective when combined with CPP-ACP products.

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