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Effects of Fractional Carbon Dioxide Laser and CPP-ACP Paste on Remineralization and Discoloration of Enamel White Spot Lesions

Nima Baniasad [1], Maryam Poosti [2], Shahrzad Etemadi [1], Farhang Mahmoudi [1], Kamal Mirmohammadi [1]

[1] Department of Orthodontics, Faculty of Dentistry, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.
[2] Private Orthodontic Practice, Tehran, Iran.

Corresponding author:
Shahrzad Etemadi, Department of Orthodontics, Faculty of Dentistry, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

etemadi.shahrzad@gmail.com

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Abstract

Background and Aim: The prevalence of enamel demineralization around orthodontic brackets is high. This study investigated the effects of CO_2 laser and casein phosphopeptide amorphous calcium phosphate (CPP-ACP) on enamel white spot lesions (WSLs).

Materials and Methods: In this in vitro study, 60 human premolars were stored in a demineralizing solution for 12 weeks to induce WSLs, and divided into four groups (n=15) of no surface treatment (control), CPP-ACP paste application for 4 minutes/day for one week, and CO_2 laser (10 mJ, 200 Hz, 10 s) with/without CPP-ACP paste. The teeth were then immersed in artificial saliva for 90 days while being subjected to daily fluoride mouthwash and weekly brushing. Tooth color was measured at baseline, after demineralization, after the interventions, and after 90 days of storage. The Vickers microhardness of the teeth was measured at the enamel surface and 30-, 60-, and 90-µm depths. Data were analyzed by one-way and repeated measures ANOVA and Friedman test.

Results: No significant difference was found among the four groups concerning color change, and all groups had clinically detectable discoloration after remineralization. Laser irradiation through CPP-ACP paste caused a significant increase in microhardness compared to CPP-ACP alone and the control group ($P<0.05$). Microhardness at 30-, 60- and 90-µm depths was also significantly greater in laser/CPP-ACP compared to other groups ($P=0.0001$).

Conclusion: Application of fractional CO_2 laser with/without CPP-ACP paste was not effective in improving the color of WSLs. However, application of CO_2 laser through CPP-ACP may be suggested for rehardening of demineralized enamel.

Keywords: Dental Enamel; Dental Caries; Tooth Remineralization; Lasers; Colorimetry; Hardness Tests; Casein Phosphopeptide-Amorphous Calcium Phosphate Nanocomplex

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Introduction

Enamel demineralization around orthodontic brackets is a common clinical problem in orthodontic treatment [1]. According to the literature, the prevalence of primary carious lesions at the end of orthodontic treatment

ranges from 50% to 93%. During orthodontic treatment, plaque accumulation around orthodontic brackets and failure to achieve proper oral hygiene can lead to enamel demineralization within a few weeks [2].

Fluoride-containing oral mouthwashes [3], fluoride varnishes [4], adhesive fluoride [5], and recently, laser application [6, 7] have been recommended to prevent enamel demineralization. Nonetheless, enamel demineralization and white spot lesions (WSLs) still occur in many patients, leading to unesthetic results and increasing the risk of carious lesions. Various approaches have been proposed to treat demineralized enamel, including topical fluoride application [8,9], fluoride-containing toothpastes [10,11], casein phosphopeptide amorphous calcium phosphate (CPP-ACP) [12], and nano-hydroxyapatite [13]. Topical application of CPP-ACP has been shown to be advantageous in remineralization of primary carious lesions. CPP is the main milk protein. CPP-ACP nanocomplex is composed of milk proteins, casein, calcium, and phosphate. The cariostatic activity of this compound has been well documented [12]. Laser application has been recommended as an effective treatment modality for primary enamel lesions [14]. Several mechanisms have explained the improvement of caries resistance after laser irradiation. The suggested mechanisms include reducing the solubility of enamel hydroxyapatite crystals, increasing the sedimentation of surface fluoride, and conversion of hydroxyapatite crystals to fluorapatite [15-17].

A study conducted in 2017 [18] revealed positive influence of CO_2 laser on microhardness of demineralized enamel, although CPP-ACP paste showed no efficacy for prevention of demineralization. Another study; however, showed superior effects of CPP-ACP fluoride varnish for prevention of enamel demineralization compared to CO_2 laser [19].

In addition, it is not clear if simultaneous application of laser and fluoridated products can improve remineralization or not. Poosti et al. [14] suggested that presence of such products may limit the rehardening effect of laser, although this negative effect has not been mentioned in other studies, as Schmidlin et al. [20], and Tepper et al. [21] suggested laser irradiation through an amine fluoride solution. Considering the suggested efficacy of CPP-ACP paste and CO_2 laser for prevention of enamel demineralization [6, 7, 12], the increase in enamel fluoride uptake after CO_2 laser irradiation [21, 22], and also the aforementioned controversies, the aim of the present study was to determine the effects of CO_2 laser compared to CPP-ACP paste on remineralization of WSLs and enamel color improvement.

Materials and Methods

This in vitro study used 60 human premolar teeth extracted for orthodontic purposes. The study protocol was ethically approved by the Research Council, Dental Faculty of Islamic Azad University (RES.CCL.DENT.IAU.2016.T/106). Teeth with cracks, attrition, discoloration, apparent hypoplasia, and hypocalcification were excluded from the study. The teeth were stored in 0.1% thymol solution, and the surface of each tooth was coated with 2 layers of acid-resistant nail varnish, except for a 4 x 4 mm^2 window at the center of the buccal surface. Each tooth was mounted at the center of a plastic cylinder 15 mm in diameter and 30 mm in height, filled with acrylic resin in a way that the tooth crown was completely out of the acrylic resin, and only the root was embedded in resin. The acrylic cylinders were marked with a bur to ensure reproducible positioning of the teeth in putty jigs during colorimetry [14].

Primary colorimetry (T_1) was performed by a spectrophotometer (Shade Pilot, Degudent,

Germany) according to the CIE L*a*b* color system, in which L* represents lightness, a* represents the saturation of green and red (a+ = red, a- = green) and b* represents the saturation of blue and yellow (b+ = yellow, b- = blue). Jigs were fabricated from putty (Zeta Plus, Zhermack, Italy) for each tooth to facilitate reproducible positioning for colorimetry in four steps [14].

Colorimetry was performed between 10 AM to 13 PM to minimize the effect of ambient light on the results. After the primary colorimetry, each tooth was separately stored in 10 cc of a demineralizing solution containing 50 mM acetic acid, 2.2 mM $CaCl_2$, and 2.2 mM NaH_2PO_4 [23] synthesized at Tehran University of Medical Sciences, Faculty of Pharmacy. The solutions were refreshed every 5 days. After rinsing the samples with distilled water for 10 seconds, colorimetry was repeated for each sample at the same point of the primary colorimetry (T_2). Subsequently, the samples were randomly divided into 4 groups (15 samples in each group) and treated as follows:

(I) No treatment control group

(II) Samples treated with CPP-ACP paste (MI paste, GC, USA): Enamel was coated with the paste for 4 minutes, and then the paste was removed with a wet gauze, rinsed with distilled water, and the procedure was repeated for 7 days. During this 7-day period, the samples were stored in saline (CPP-ACP group).

(III) CO_2 laser group: Samples were irradiated with fractional CO_2 laser with 10.6 µm wavelength (Lutronic Inc., Princeton, USA), 200 Hz frequency, 10 mJ energy, 10 W power, and 1 mm tip diameter. The laser's handpiece was in direct contact with the enamel surface. The samples underwent irradiation for 10 seconds [14].

(IV) Laser + CPP-ACP group: The samples were simultaneously treated with laser and CPP-ACP paste. Paste was applied on the enamel, and after 1 minute, laser irradiation with the same parameters as in group 3 was performed for 10 seconds. Then, the paste remained on the sample surface for an additional 3 minutes before rinsing. After the interventions, colorimetry was repeated (T3). Subsequently, the samples underwent a 90-day remineralization period simulating at-home remineralization. They were stored in artificial saliva at 37°C and rinsed daily with 0.05% fluoride mouthwash (Oral-B Advantage; UK) and brushed weekly by Oral-B Cross-Action electric toothbrush and Sensodyne toothpaste containing 1400 ppm fluoride. Artificial saliva contained 0.4 g/L KCl, 0.4 g/L NaCl, 0.906 g/L $CaCl_2.2H_2O$, 0.690 g/L NaH2PO4.2H2O, 0.005 g/L Na_2S9H_2O, and 1 g/L urea with a pH of 7.03. This solution was changed daily. The final colorimetry was then performed (T_4). The color change (ΔE) of each sample over the course of treatment was calculated using the following formula [14]:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

A ΔE more than 3.3 units showed a clinically detectable color mismatch.

In the next step, the samples were prepared for cross-sectional microhardness testing in order to evaluate enamel hardness. First, the roots were cut, and the crowns were sectioned occlusogingivally into two halves through the center of the enamel window using a low-speed diamond disc. After polishing the surfaces with abrasive papers, a microhardness tester (Matsuzawa, Japan) was employed to measure the Vickers microhardness number. For this purpose, the device indenter was placed on the tooth surface, and at 30-, 60-, and 90-µm depths, and a vertical force of 100 g was applied for 10 seconds. Then, the microhardness value was measured based on the dimensions of the created indentation [14].

Statistical analysis:

Statistical analysis was performed using SPSS version 22. The Kolmogorov–Smirnov test was performed to evaluate the normality of data distribution. One-way ANOVA was utilized to compare ΔE among the four groups at selected time points, and microhardness values among

the groups at each depth. The Bonferroni test was employed for pairwise comparisons. P<0.05 was considered statistically significant.

Results

Color assessment:

Table 1 compares the color parameters (L*, a*, b*) among the four study groups. As shown, a significant difference was observed among the groups only in b* parameter at the enamel surface in the treatment stage and after remineralization (P= 0.007 and P= 0.0001, respectively). At both the aforementioned time points, the b* parameter was higher in the laser/CPP-ACP group than the other three groups as shown by one-way AVOVA.

Table 2 shows the ΔE at different time points [$\Delta E(T_1\text{-}T_2)$, $\Delta E(T_2\text{-}T_3)$, $\Delta E(T_3\text{-}T_4)$, $\Delta E(T_1\text{-}T_4)$]. According to repeated measures ANOVA, there was no significant difference in ΔE at different time points (P=0.29). In addition, at each time point ($T_1\text{-}T_2$, $T_2\text{-}T_3$, $T_3\text{-}T_4$, $T_1\text{-}T_4$), one-way ANOVA showed no significant difference in ΔE among the four groups (P=0.35, P=0.92, P=0.267, and P=0.232, respectively). It is worth mentioning that when comparing the baseline ΔE with the final ΔE [$\Delta E(T_1\text{-}T_4)$], the values were clinically higher than the acceptable value (i.e., 3.3) in all groups [$\Delta E(T_1\text{-}T_4)>3.3$]. However, the highest ΔE value was observed in the control group equal to 5.43±1.12, and the lowest was recorded in the laser + CPP-ACP group equal to 4.51±1.6. In addition, in the laser + CPP-ACP group, 4 samples were observed with an acceptable $\Delta E(T_1\text{-}T_4) < 3.3$; among which, the lowest value was 2.2. However, in the control group, none of the samples had acceptable ΔE, while two samples in the CPP-ACP group and two samples in the laser group had ΔE>3.3.

Microhardness:

Table 3 compares microhardness of the surface and at 30-, 60-, and 90-μm depths in the four groups. One-way ANOVA showed a significant difference in microhardness values at different sites (P=0.002 at the surface, P=0.001 at 30 μm, P=0.0001 at 60 μm, and P=0.002 at 90 μm depth). The highest microhardness values were observed in the laser + CPP-ACP group on the surface and at 30, 60, and 90 μm depths, while the lowest microhardness values were found in the control group on the surface and at 30, 60, and 90 μm depths.

In addition, when considering each group separately, the Friedman test revealed a significant difference in microhardness values on the surface and at 30, 60, and 90 μm depths (P=0.001 at the surface, P=0.001 at 30 μm, P=0.013 at 60 μm, and P=0.0001 at 90 μm depth). The lowest microhardness value was found at 30 μm depth in the control group, and at the enamel surface in the remaining three groups. In all four groups, the highest microhardness value was observed at 90 μm depth.

Table 4 shows pairwise comparisons of the groups regarding the microhardness values at each site. The results showed that on the surface, the microhardness value in the control group was significantly lower than that in the laser + CPP-ACP group (P=0.002). Furthermore, at the same site, the microhardness value was significantly lower in the CPP-ACP group than in the laser + CPP-ACP group (P=0.01).

At 30 μm depth, the microhardness value was significantly higher in the laser + CPP-ACP group compared to the control group and also the CPP-ACP group (P=0.002).

At 60 μm depth, the microhardness value was significantly higher in the laser + CPP-ACP group compared to the control group (P=0.0001), CPP-ACP group (P=0.006), and also the laser group (P=0.035).

At 90 μm depth, the microhardness value was significantly higher in the laser + CPP-ACP group compared to the control group (P=0.004), CPP-ACP group (P=0.017), and also the laser group (P=0.019).

Table 1. Colorimetry parameters (L*, a*, b*) in the study groups

		Group					P-value*
		Control	CPP-ACP	Laser	laser + CPP-ACP	Total	
L1	Mean	83.37	83.03	83.2	82.68	83.1	0.869
	Standard Deviation	2.65	2.99	1.47	1.75	2.26	
	Minimum	79.5	75.4	80.2	79.8	75.4	
	Maximum	89.3	87.4	85.2	86	89.3	
a1	Mean	8.56	8.74	8.35	8.93	8.65	0.95
	Standard Deviation	2.47	3.26	2.93	2.51	2.75	
	Minimum	5.5	3.7	3.5	6.1	3.5	
	Maximum	13.6	16.8	13.3	14.3	16.8	
b1	Mean	10.23	12.01	12.3	12.41	11.8	0.057
	Standard Deviation	1.65	3.12	2.55	2.21	2.54	
	Minimum	7.7	7.5	7.5	9.3	7.5	
	Maximum	13.6	17	16.5	16.9	17	
L2	Mean	80.17	79.7	80.1	78.95	79.7	0.563
	Standard Deviation	2.43	3.27	2.53	2.11	2.6	
	Minimum	76.4	72	75	75.1	72	
	Maximum	84.7	84.3	84.7	83.2	84.7	
a2	Mean	6.25	5.99	5.25	6.05	5.89	0.669
	Standard Deviation	2.53	2.83	2.18	1.77	2.33	
	Minimum	0.7	0	0	3.1	0	
	Maximum	10.1	13.1	8.5	9	13.1	
b2	Mean	6.39	8.05	7.59	9.17	7.8	0.019
	Standard Deviation	2.04	2.63	2.39	2.34	2.51	
	Minimum	3.5	5.1	4.5	5.7	3.5	
	Maximum	10.8	14.9	12.2	13.2	14.9	
L3	Mean	.	79.05	78.7	78.21	78.7	0.689
	Standard Deviation	.	3.23	2.47	2.21	2.63	
	Minimum	.	71.8	73.9	74.5	71.8	
	Maximum	.	83.7	82.5	82.9	83.7	
a3	Mean	.	5.77	4.11	4.45	4.77	0.125
	Standard Deviation	.	3.11	2.09	1.34	2.36	
	Minimum	.	-0.2	1.3	3	-0.2	
	Maximum	.	13.7	8.3	7.8	13.7	
b3	Mean	.	8.44	8.77	11.05	9.42	0.007
	Standard Deviation	.	2.71	2.03	2.18	2.56	
	Minimum	.	3.9	5.7	7.9	3.9	
	Maximum	.	13.9	12	15.6	15.6	
L4	Mean	80.01	81.39	81.2	80.15	80.7	0.282
	Standard Deviation	2.03	3.13	2.09	2.23	2.43	
	Minimum	76.4	74.1	78.1	76.8	74.1	
	Maximum	83.7	86.7	84.5	85.1	86.7	
a4	Mean	6.35	6.69	5.5	6.01	6.14	0.519
	Standard Deviation	2.25	3.08	1.83	1.51	2.23	
	Minimum	1.7	0.8	2.5	4.3	0.8	
	Maximum	10.2	14.5	8.6	9.3	14.5	
b4	Mean	7.3	9.77	9.88	11.43	9.6	0.0001
	Standard Deviation	1.67	2.26	1.92	2.07	2.45	
	Minimum	5.1	7.2	7.2	8.1	5.1	
	Maximum	10.4	15.3	13.1	15.6	15.6	
P value							

*: P value calculated based on one-way ANOVA

Table 2. ΔE of the study groups at different time points

Variable		Group					P value*
		Control	CPP-ACP	Laser	laser + CPP-ACP	Total	
ΔE1-2	Mean	6.01	6.63	7.05	6.23	6.48	0.35
	Standard Deviation	1.47	2.14	1.41	1.64	1.70	
	Minimum	4.20	3.90	4.80	4.00	3.90	
	Maximum	9.40	12.90	9.00	9.00	12.90	
ΔE2-3	Mean	.	2.34	3.01	2.97	2.77	.092
	Standard Deviation	.	1.09	0.75	0.84	0.93	
	Minimum	.	1.20	1.90	1.60	1.20	
	Maximum	.	4.70	4.60	4.60	4.70	
ΔE3-4	Mean	.	3.09	3.29	2.77	3.05	0.267
	Standard Deviation	.	0.95	0.94	0.65	0.87	
	Minimum	.	1.30	1.50	1.50	1.30	
	Maximum	.	4.70	4.50	3.80	4.70	
ΔE1-4	Mean	5.43	4.55	4.85	4.51	4.84	0.232
	Standard Deviation	1.12	1.53	1.14	1.60	1.38	
	Minimum	3.60	2.60	3.10	2.20	2.20	
	Maximum	6.80	8.80	7.00	8.00	8.80	
P value**				0.29			

*: P value calculated based on one-way ANOVA

**: P value calculated based on repeated measures ANOVA

Table 3. Microhardness values in the study groups

Microhardness		Group				
		Control	CPP-ACP	Laser	Laser + CPP-ACP	P value*
Enamel surface	Mean	278.43	282.39	288.05	308.13	.002
	Standard Deviation	22.24	21.43	19.90	21.52	
	Minimum	224.00	240.00	255.00	270.00	
	Maximum	310.00	323.00	326.80	352.30	
30 µm depth	Mean	275.19	285.05	296.51	318.64	.0001
	Standard Deviation	23.28	18.56	30.34	22.68	
	Minimum	226.00	251.00	254.40	284.50	
	Maximum	315.70	326.30	376.00	359.24	
60 µm depth	Mean	271.35	288.25	293.92	319.74	.0001
	Standard Deviation	28.46	17.14	29.05	21.90	
	Minimum	205.00	263.00	260.30	281.00	
	Maximum	313.00	326.30	369.00	362.10	
90 µm depth	Mean	291.58	296.45	296.74	325.45	.002
	Standard Deviation	21.60	17.26	33.22	26.83	
	Minimum	243.00	271.00	261.40	281.00	
	Maximum	324.00	340.40	384.00	383.10	
P value **	0.0001		0.0001	0.013	0.0001	

*: P value calculated based on one-way ANOVA

**: P value calculated based on Freidman test

Table 4. Pairwise comparisons of the groups regarding microhardness values with the Bonferroni test

Dependent Variable	Group (I)	Group (J)	Mean Difference (I-J)	Std. Error	P value
Enamel surface	Control	laser + CPP-ACP	-29.7	7.77416	0.002
	CPP-ACP	Laser + CPP-ACP	-25.74667	7.77416	0.01
30 µm depth	Control	Laser + CPP-ACP	-43.44933	8.79667	0.0001
	CPP-ACP	Laser + CPP-ACP	-33.58933	8.79667	0.002
60 µm depth	Control	Laser + CPP-ACP	-48.39333	8.99406	0.0001
	CPP-ACP	Laser + CPP-ACP	-31.48667	8.99406	0.006
	Laser	Laser + CPP-ACP	-25.82	8.99406	0.035
90 µm depth	Control	Laser + CPP-ACP	-33.86667	9.28677	0.004
	CPP-ACP	Laser + CPP-ACP	-28.99333	9.28677	0.017
	Laser	Laser + CPP-ACP	-28.70667	9.28677	0.019

Discussion

Enamel discoloration is one of the side effects of orthodontic treatment that causes patient dissatisfaction [24]. Approximately, 49.6% of patients experience demineralization and tooth discoloration after orthodontic treatment [25]. In the current study, the effect of fractional CO_2 laser and CPP-ACP paste on induced WSLs was investigated, focusing on color change and microhardness as enamel remineralization indices. The purpose of this study was to find an appropriate solution to improve the appearance and hardness of demineralized enamel in order to reduce the surface porosity as a consequence of orthodontic treatment. Based on the results, none of the tested treatments, namely CPP-ACP paste application, CO_2 laser irradiation, and combination of both, had a significant effect on discoloration of WSLs. However, when assessing microhardness as a remineralization index, samples in the laser + CPP-ACP group showed the highest microhardness value, indicating the positive effects of this intervention on the microhardness of WSLs. It is worth mentioning that the difference between the efficacy of laser + CPP-ACP group and the laser group was at deeper sites (i.e., 60 µm and 90 µm depths), suggesting that laser irradiation may be crucial to prevent demineralization at deeper lesion sites.

Poosti et al. [14] compared the impact of CO_2 laser and acidulated phosphate fluoride gel (APF) on enamel WSLs in terms of microhardness and color change. The study also evaluated the effects of laser application before and through APF gel. The results indicated that laser application through the APF gel might have negative effects on the color of demineralized enamel. In contrast, the present study did not show such negative effects on color parameters in laser + CPP-ACP group. In addition, Poosti et al. [14] reported lower color difference between baseline (T1) and final (T4) examination when laser irradiation occurred before or through APF gel application compared to APF alone and control groups. The present results also indicated the lowest color change between T1 and T4 in the laser + CPP-ACP group, although the difference was not statistically significant. Laser causes microscopic pores within the structure of irradiated enamel, which results in mineral absorption during the 90-day remineralization period. In terms of microhardness, at 30 and 60 µm depths, laser irradiation before APF gel application showed significantly greater results compared to other groups, which means that laser irradiation through APF was not effective for microhardness improvement. The study suggests that presence of APF may inhibit temperature rise caused by CO_2 laser, which limits the rehardening effect of laser. However, in the present study, the laser + CPP-ACP group showed the highest microhardness on the surface and at 30, 60, and 90 µm depths. However, the present study did not evaluate laser irradiation before CPP-ACP paste application, which could be considered a limitation of the present study.

It is believed that laser irradiation causes ultrastructural changes, such as the growth of crystal size because of temperature rise, which could be responsible for its effect on enamel microhardness [26, 27]. However, there are controversial results regarding whether laser irradiation should be performed before, during, or after fluoride treatment. Tagomori and Morioka [28] recommended irradiation before fluoride application; whereas, Schmidlin et al. [20], and Tepper et al. [21] suggested laser irradiation through an amine fluoride solution. Farhadian et al. [18] conducted a study aiming to evaluate the effect of CPP-ACP paste and CO_2 laser irradiation on demineralized enamel microhardness and shear bond strength of orthodontic brackets. Comparison of Vickers microhardness of different groups revealed that

only CO_2 laser showed a significant difference with the control group revealing insignificant efficacy of CPP-ACP paste for demineralization prevention. In addition, laser irradiation alone caused a higher microhardness value than CO_2 laser irradiation either before or through CPP-ACP application, which was in contrast to the results of the present study. Similar to the study by Poosti et al. [14], it is postulated that CPP-ACP prevents the temperature rise induced by laser irradiation, resulting in no synergistic effect. Further studies are needed to evaluate the effect of laser application before, during, and after CPP-ACP paste application.

Yassaei and Motallaei [29] evaluated the effect of Er:YAG laser and MI Paste Plus on WSLs and reported that Er:YAG laser irradiation before MI Paste Plus application resulted in the highest microhardness rate at all studied depths. However, comparing with the results of other groups, the difference was statistically significant at 50 and 150 µm depths. Application of Er:YAG laser or MI Paste Plus alone did not show statistically significant results. Similarly, in the present study, CO_2 laser or CPP-ACP paste alone did not show significantly better effects on microhardness. MI Paste Plus contains CPP-ACP and fluoride (900 ppm) and is believed to have a superior remineralizing effect on enamel lesions compared to CPP-ACP or sodium fluoride alone [30-32]. High power lasers, such as Er:YAG, can induce cracks and roughen the enamel structure, making teeth susceptible to caries, especially in demineralized enamel [33-35]. The failure of Er:YAG laser irradiation alone for remineralization in the present study may be attributed to the negative effects of laser alone. Some previous studies [36, 37] reported positive results of CPP-ACP and CPP-ACPF (MI Paste Plus) on remineralization of WSLs. However, they used these compositions for 1 to 3 months with a high frequency. In the present study, similar to the study by Yassaei and Motallaei [29,] limited duration of CPP-ACP and MI Paste Plus application may have been responsible for the results, showing that their application alone, without a combined laser treatment, does not have significant positive effects on remineralization of enamel WSLs.

Abufarwa et al. [19] compared the area, intensity, and impact of demineralization, and microhardness in samples treated separately with CPP-ACP fluoride varnish and CO_2 laser, concluding that CPP-ACP fluoride varnish is more effective than CO_2 laser in preventing enamel demineralization. MI varnish (fluoride-containing CPP-ACP) used in this study has 22000 ppm fluoride, and the increased enamel hardness can be attributed to the synergistic effect of fluoride and CPP-ACP complex [38]. Abufarwa et al. [19] reported that MI varnish increased enamel hardness to 60 µm depth, but CO_2 laser failed to yield encouraging results, which may be due to the laser parameters used. More studies are required to find appropriate laser parameters for prevention of enamel WSLs.

Conclusion

CPP-ACP paste and fractional CO_2 laser, neither alone nor in combination, did not improve the appearance of enamel WSLs compared to the control group. CO_2 laser irradiation through CPP-ACP paste increased microhardness at 30, 60, and 90 µm depths. CO_2 laser or CPP-ACP paste alone cannot enhance enamel remineralization in a long-term remineralization procedure.

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