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ORIGINAL ARTICLE

Effect of CO_2 laser (10.6 μm) and Remin Pro on microhardness of enamel white spot lesions

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Abstract
This study investigated the combined effect of CO_2 laser irradiation and Remin Pro paste on microhardness of enamel white spot lesions (WSLs). Seventy-eight intact premolars were randomly assigned into six groups and then stored in a demineralizing solution to create WSLs. Afterwards, the teeth in group 6 (negative control) remained untreated, while groups 1 and 4 were exposed to CO_2 laser irradiation (20 Hz, 1 W, 30 s) and Remin Pro paste, respectively. In groups 2 and 3, the teeth were exposed to laser either before (group 2) or after (group 3) Remin Pro application. The teeth in groups 1 to 5 were then immersed in artificial saliva for 90 days while subjected to fluoride mouthwash and weekly brushing. Finally, the teeth were sectioned, and Vickers microhardness was measured at the enamel surface and at 50, 100, and 150 μm from the surface. One sample of each group was also examined with scanning electron microscope (SEM). Data were analyzed by two-way analysis of variance (ANOVA) and Tukey's test. The significance was set at 0.05. Laser irradiation followed by Remin Pro application (group 2) caused a significant increase in total WSLs' microhardness compared with laser alone (group 1) and control groups ($P < 0.05$). Microhardness at depths of 100 and 150 μm was also significantly greater in group 2 compared with those of group 3 and control groups ($P < 0.05$). Combined application of CO_2 laser with Remin Pro paste, when laser is irradiated before the paste, is suggested for re-hardening of WSLs in deep layers of enamel.

Keywords CO_2 laser · Tooth remineralization · White spots

Introduction

The risk of creating white spot lesions (WSLs) on the enamel surface is one of the complications that can compromise the esthetic benefits of orthodontic treatment [1]. WSLs are the first macroscopic sign of enamel caries in which, the external layer of enamel usually stays intact while the underlying layers are demineralized, and in the absence of treatment, it becomes cavitated [2]. Prevalence of WSLs in different articles has been reported between 2 and 97% [1, 3–6] and in some studies between 50 and 70% [7].

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Tooth demineralization in the primary levels can be remineralized naturally by saliva containing calcium, phosphorus and fluoride ions, buffering compounds, and other substances [8]. However, natural remineralization by saliva does not improve the esthetic and structural properties of the deeper lesions as much [5]. Complete removal of enamel WSLs is difficult [9], and some lesions remain 5–12 years after the end of treatment. Therefore, the use of remineralizing agents for the restoration of deeper lesions and achieving better esthetics is essential [10].

Remin Pro (VOCO, Germany) is one of the newest water-based remineralizing toothpastes that contains calcium phosphate in the form of hydroxyapatite, fluoride (1450 ppm), and xylitol. Hydroxyapatite replaces the lost enamel; fluoride seals the dentinal tubules; and xylitol acts as an antibacterial agent. This material has been recommended for controlling tooth sensitivity, preventing enamel demineralization, enamel remineralization stimulation, and being used in orthodontic patients [11, 12].

Due to shortages and disadvantages of existing methods for prevention and treatment of WSLs, new methods have been discussed. These include high-power laser irradiation, such as neodymium-doped yttrium aluminum garnet (Nd: YAG) laser, argon laser, and carbon dioxide (CO_2) laser on the enamel.

Laser is recommended to increase tooth resistance against demineralization and WSLs treatment before fluoride therapy to restore color and hardness of demineralized enamel [13, 14]. Laser application along with fluoride compounds increases the uptake and durability of fluoride and enamel's acid resistance so that the pH required for enamel solubility decreases from 5.5 to 4.3 [15].

In the range of studies conducted on WSLs treatment methods, the combination effect of the studied factors in the present study (Remin Pro and CO_2 laser) was not found, and insufficient information is available in this regard. Therefore, the purpose of this study was to evaluate the effect of CO_2 laser and Remin Pro on remineralization of WSLs.

Materials and methods

Seventy-eight premolar teeth with intact crowns, which needed to be extracted (due to orthodontic treatment) were collected, cleaned, and then stored in saline solution at room temperature [16].

All samples were placed in 10 ml of demineralizing solution for 96 h to create artificial enamel white spots [17]. The demineralizing solution was composed of 0.05 M lactic acid, 2.2 mM calcium chloride ($CaCl_2$), 2.2 mM sodium dihydrogen phosphate (NaH_2PO_4), and 0.2 ppm fluoride. The solution was adjusted with 50% NaOH to reach pH 4.5 [18]. The solution was replaced on a daily basis. After performing the above-mentioned process, samples were washed with distilled water, and all surfaces of the teeth in the treatment groups with the exception of a 4 × 4 mm window in the center of the buccal surface as the treatment area were covered with two layers of nail varnish [16]. Samples were kept in 37 °C artificial saliva (NIK CERAM RAZI Corporation, Esfahan, Iran) for a 3-month period. This solution was also replaced on a daily basis.

The samples were then randomly divided to 6 groups, namely, group 1 (CO_2 laser irradiation) (L) ($n = 13$), group 2 (CO_2 laser irradiation + Remin Pro) (L1 + R2) ($n = 13$), group 3 (Remin Pro + CO_2 laser irradiation) (R1 + L2) ($n = 13$), group 4 (Remin Pro) (R) ($n = 13$), group 5 (negative control group) (C−) ($n = 13$), group 6 (positive control group) (C+) ($n = 13$).

In group 1, the teeth were only exposed to laser irradiation. Each time before applying the laser, samples were carefully dried with dental air spray. CO_2 laser (MultiXel (DS-40UB), DAESHIN ENTERPRISE, Seoul, South Korea) with 10.6 μm wavelength, 20 Hz frequency, output power of 1 W, pulse duration of 10 ms, pulse interval of 15 ms, and beam diameter of 0.6 mm for 30 s from a 2-mm distance with a sweeping motion was used (Fig. 1).

In group 2, the teeth were irradiated with laser, and then Remin Pro (VOCO GmbH, Cuxhaven, Germany) was applied according to the manufacturer's instructions for 3 min by an electric toothbrush (AQ-100 AQUAPICK, Whangarei, New Zealand).

In group 3, Remin Pro was applied, and then samples were subjected to laser irradiation.

In group 4, only Remin Pro was applied on samples.

In group 5, no treatment was performed, and only enamel microhardness was measured with Vickers test at the surface and depths of 50, 100, and 150 μm to ensure the creation of WSLs after demineralization. The samples were then stored in distilled water until the test was performed.

In group 6 (positive control group), samples did not receive any of the above-mentioned treatments (laser and Remin Pro) and were only kept in artificial saliva for intraoral remineralization simulation. Laser irradiation and the application of Remin Pro were performed in groups 1 to 4, once at the beginning of the process, the second time was 1 month later, and the third time was 2 months after the beginning of the study.

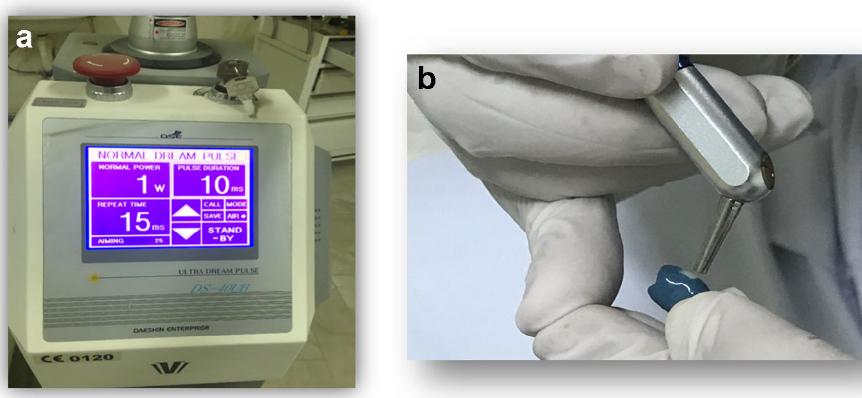


Fig. 1 CO_2 laser. **a** Laser settings. **b** Laser irradiation

After the first stage of the treatment, samples were kept in artificial saliva for 90 days, and in order to simulate intraoral remineralization, they were exposed to fluoride mouth wash (Oral-B Pro Expert, Germany) and brushing three times a week with toothpaste (Fresh Mint Crest 7 Complete) [13]. Each specimen was kept in mouthwash for 2 min and was brushed for 10 s (Fig. 2).

Vickers microhardness test

To perform the hardness test, samples were washed with distilled water and were mounted in acrylic resin. Then, the blocks were cut longitudinally into mesial and distal halves with a diamond disk and a high-speed handpiece at the center of the buccal surface. Diamond disk and a caliper were used to parallel the surfaces. The sample surfaces were polished by 400, 800, 1000, and 2000 grit silicon carbide paper sequentially to provide a smooth and standard surface for the microhardness test. Finally, in order to achieve a shiny surface to see the pressure effects of the device and measure the hardness, samples were fine polished with 220 grit polishing paste (Fig. 3).

To measure microhardness at the surface and the depths of 50, 100, and 150 μm, a microhardness test device by Vickers method (Micro Hardness Tester, FM 700 series, FUTURE-TECH CORP., Japan) was used (Fig. 4).

In this study, the loading rate was 50 g/f, and dwell time (load time) was considered to be 5 s. Hardness of samples was measured as a hardness profile. So that the hardness for each depth was measured 3 times, and the average was recorded as the microhardness value. The microhardness test was performed on 12 samples from each group.

At the end of the study, a sample of each group was examined with an electron microscope (SEM, VEGA 3, TESCAN, Czech Republic) to study the morphology of enamel surface with a magnification of × 2000. For this purpose, enamel blocks were dried completely using 100% alcohol and then were covered with a thin layer of gold.

Fig. 2 Materials used in this study. **a** Artificial saliva. **b** Crest 7 complete tooth paste. **c** Remin Pro forte. **d** Oral-B Pro Expert mouth wash

Fig. 3 Prepared enamel blocks

Data analysis

Analyzing the data and calculating means and standard deviations of microhardness in different groups and depths was done using SPSS software (version 25).

Initially, normality of the data was analyzed by Kolmogorov-Smirnov test, and normal distribution of the microhardness variables was assured. With regard to normal distribution of data, two-way ANOVA test was used. Comparison of the means between groups and the depths was studied with Tukey's test.

Results

The results of variance analysis of the obtained data indicated that the effect of study groups and different depths on the microhardness was significant ($P < 0.001$). The interaction between study groups and different depths was also significant ($P = 0.001$) (Table 1).

The obtained results were classified in to three categories:

- Comparison of total microhardness means of the study groups at the studied surfaces
- Comparison of microhardness means between the study groups at various depths
- Comparison of microhardness means of the study groups between various depths

Comparison of the microhardness means in the study groups indicated different microhardness in various study groups (Chart 1, Table 1). In other words, the maximum and minimum microhardness were measured in the Remin Pro after laser group (L1 + R2) (424.04) and negative control group (C−) (327.18), respectively.

Comparing the mean scores of each group with other study groups re

presented a significant difference between a number of them. The microhardness means of all study group was significantly higher than the negative control group ($P < 0.0\ 5$). Also, the microhardness means in group L1 + R2 was significantly higher than those of groups R1 + L2, L, C+, and C−. Also, in group R, the microhardness mean was significantly higher than those of groups R1 + L2, C+, and C−.

Comparison of the microhardness means of study groups at various depths represented the most significant differences between the mentioned groups in all depths (Table 2). In other words, many study groups in each depth had significant differences with other groups in the same depth. For example, the highest and lowest microhardness at the depth 0 (surface) was observed in the group R (444.02) and C− (281.15), respectively (Chart 2a). At this depth, all the study groups showed a significantly higher microhardness value than group C−. At the depth of 50 μm, the microhardness means in groups L1 + R2 and R were more than other groups and had a significant difference with group C− (Chart 2b). Maximum microhardness at 100 μm depth was observed in group L1 + R2 that was significantly higher than groups

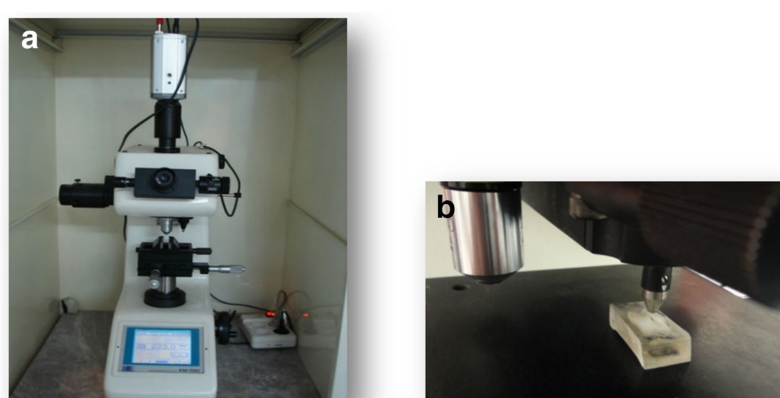


Fig. 4 Microhardness test. **a** Microhardness tester. **b** A sample under the microhardness tester

Table 1 Comparison of the total microhardness means of study groups at the studied surfaces

Study groups	Microhardness means (MicroVickers)	Standard error	Pairwise comparisons	95% confidence interval	
				Lower bound	Upper bound
C−	327.18	7.39	a	312.61	341.75
C+	368.99	7.39	d	354.42	383.55
R	413.33	7.72	c, e	398.12	428.55
L	392.88	7.39	d, e	378.31	407.45
R1 + L2	370.47	7.39	d	355.90	385.04
L1 + R2	424.04	7.72	b, c	408.83	439.26

The means with similar letters did not have a significant difference

C−, control; C+, artificial saliva; R, Remin Pro®; L, CO2 laser; R1 + L2, Remin Pro® + CO_2 laser; L1 + R2, CO_2 laser + Remin Pro®

R1 + L2, C+, and C−. Also, at this depth, the group R showed a significantly higher microhardness compared with group C− (Chart 2c). At the depth of 150 μm, the maximum microhardness was also seen in group L1 + R2, which was significantly higher than groups R1 + L2, C+, and C− (Fig. 2d).

Comparison of the microhardness means of each study group in various depths are shown in Table 3. The highest microhardness in all groups was observed at the depth 0 (surface) except for the negative control group, and with increasing the depth, microhardness was reduced (Chart 3). However, the reduction process was different in various groups so that the microhardness mean difference between various depths was significant only in groups C−, R, and R1 + L2. In other words, the microhardness means of a depth of 150 μm with a depth of 50 μm had a significant difference in the group R. While in the samples of group R1 + L2, microhardness mean was significant between the depth of 0 (surface) and the depths of 100 and 150 μm, but other differences in this group were not statistically significant. Also, in the samples of group C−, the surface microhardness mean was significantly lower than those of other depths.

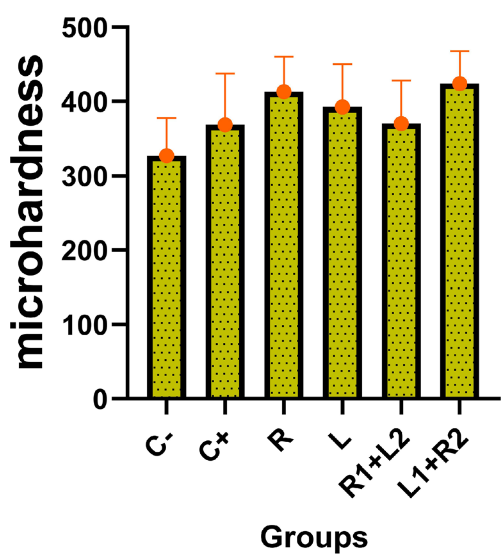


Chart 1 Means microhardness of study groups

SEM analysis

At the end of the study, a sample of each group was examined with a scanning electron microscope (SEM) to study the morphology of enamel surface with a magnification of × 2000. The SEM images from different groups had differences in the enamel morphology (Fig. 5). The images showed the creation of cracks and fine scrapes in groups with laser irradiation. Also, the SEM images of treated surfaces with Remin Pro showed a globular pattern of minerals.

Discussion

One of the complications that can compromise various benefits of orthodontic treatment, especially esthetics, is WSLs [1]. For the prevention or treatment of the mentioned lesions, several methods have been suggested among which the most important ones are the use of fluoride-containing compounds, remineralizing pastes, vital tooth bleaching, resin, indirect restorations, and laser [13, 19, 20]. However, few studies have evaluated the combined use of these methods and their results. In the present study, the combination use of CO_2 laser and Remin Pro was investigated. The criterion for assessing the effect of each of the therapeutic interventions was the final microhardness.

There are several methods to measure the amount of enamel demineralization and compare its different degrees, in which visual examination, quantitative light-induced fluorescence, the use of polarizing microscope, microradiography,

Table 2 Comparison of the microhardness means between study groups in different depths

	Surface			Depth 50			Depth 100			Depth 150		
Depth group	Mean	SD	Pairwise comparisons	Mean	SD	Pairwise comparisons	Mean	SD	Pairwise comparisons	Mean	SD	Pairwise comparisons
C− (*n* = 12)	281.15	51.28	b	353.22	60.79	b	338.95	35.48	c	335.40	16.79	a
C+ (*n* = 12)	400.95	68.66	a	376.94	73.44	a,b	358.12	66	b,c,d	339.94	59.05	a,c
R (*n* = 12)	444.02	53.58	a	427.66	42.65	a,c	402.08	39.55	a,b,d	379.58	21.69	a,b
L (*n* = 12)	417.31	58.63	a	401.56	56.62	a,b	385.61	65.96	a,b,c	367.04	51.16	a,b
R1 + L2 (*n* = 12)	417.85	41.39	a	379.20	47.66	a,b	348.20	41.29	b,c	336.61	64.50	a,c
L1 + R2 (*n* = 12)	442.38	45.25	a	423.11	41.95	a,c	423.54	43.92	a	407.14	42.15	b
P value	< 0.001*			0.013*			< 0.001*			0.002*		

Means with similar letters in each column do not have a significant difference

*Significant at level 5% (*P* value <0.05)

C−, control; C+, artificial saliva; R, Remin Pro®; L, CO_2 laser; R1 + L2, Remin Pro® + CO_2 laser; L1 + R2, CO_2 laser + Remin Pro®

and microhardness tests are among the most important of them. Since enamel hardness is linked with its amount of minerals and enamel demineralization is associated with mineral loss, microhardness test is widely used to determine the amount of enamel demineralization in laboratory studies [21, 22]. Vickers and Knoop microhardness tests are among the methods that have extensive use in dentistry. However, for assessment of the enamel microhardness in small areas, the Vickers hardness method is more suitable [23, 24]. On the other hand, Knoop test requires a lot of polish that might cause errors, while because of the lower sensitivity of Vickers test to sample's surface condition, not much polish is needed [25]. Other benefits of the Vickers test that can be noted are high accuracy, using one type of indenter for various materials and methods, and the ability to test a variety of materials under various forces [26]. In this study, the Vickers microhardness

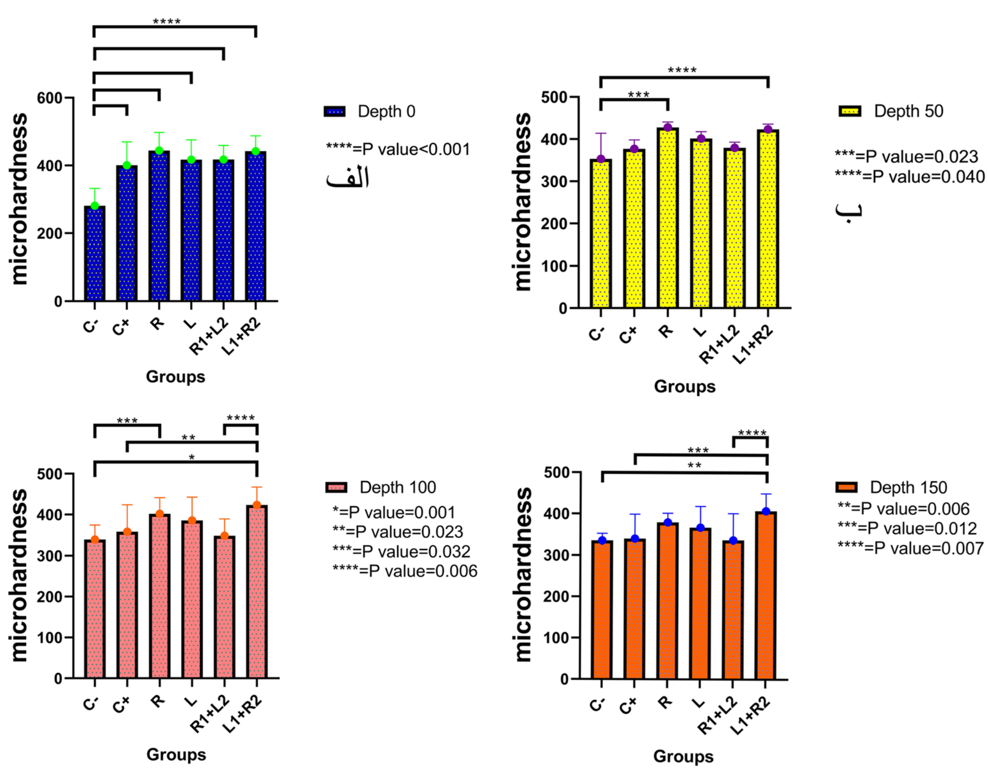


Chart 2 Comparison of the mean microhardness of study groups in each depth. **a** Surface. **b** Depth of 50 μm. **c** Depth of 100 μm. **d** 150 μm depth

Table 3 Comparison of the microhardness means of each study group at different depths

Surface				Depth 50			Depth 100			Depth 150			*P* value
Depth group	Means	SD	Pairwise comparisons	Means	SD	Pairwise comparisons	Means	SD	Pairwise comparisons	Means	SD	Pairwise comparisons	
C− ($n = 12$)	281.15	51.28	a	353.22	60.79	b	338.95	35.48	b	335.40	16.79	b	0.003*
C+ ($n = 12$)	400.95	68.66	a	376.94	73.44	a	358.12	66	a	339.94	59.05	a	0.157
R ($n = 12$)	444.02	53.58	a,c	427.66	42.65	a,c	402.08	39.55	a,b	379.58	21.69	b	0.004*
L ($n = 12$)	417.31	58.63	a	401.56	56.62	a	385.61	65.96	a	367.04	51.16	a	0.165
R1 + L2 ($n = 12$)	417.85	41.39	b	379.20	47.66	a,b	348.20	41.29	a,c	336.61	64.50	a,c	0.001*
L1 + R2 ($n = 12$)	442.38	45.25	a	423.11	41.95	a	423.54	43.92	a	407.14	42.15	a	0.316

Means with similar letters in each column do not have a significant difference

*Significant at level 5% (*P* value < 0.05)

C−, control; C+, artificial saliva; R, Remin Pro®; L, CO_2 laser; R1 + L2, Remin Pro® + CO_2 laser; L1 + R2, CO_2 laser + Remin Pro®

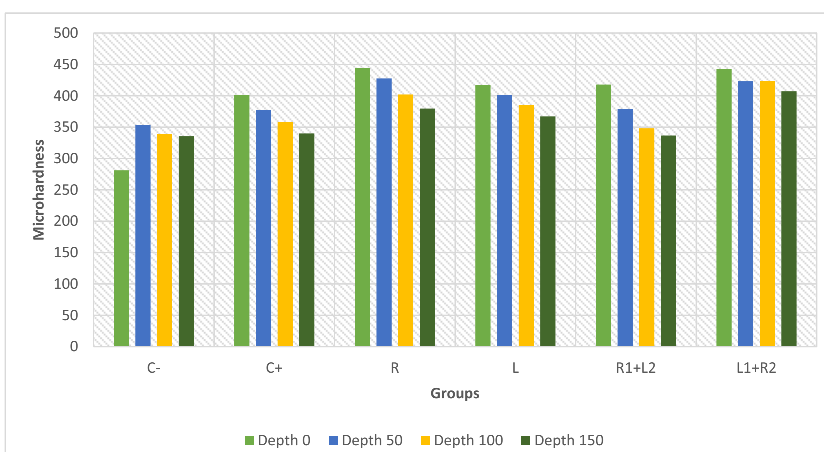
test was used as a quantitative method for measuring the amount of enamel demineralization.

In order to determine the sample hardness profile, hardness is measured in several depths. However, in different studies, various intervals have been used as an indentation place [27–29]. In this study, the first test site was chosen at the surface, and in order to avoid indentation super imposition, indentation intervals were 50 μm from each other [27]. In general, the enamel hardness in all groups showed a reduction from the surface to the depth except in the negative control group. In other words, the destructive effect of demineralizing solution and the therapeutic effect of interventions were higher at the surface. At the surface, all groups with therapeutic interventions as well as the positive control group showed significantly higher microhardness measures than the negative control group which represents the effectiveness of therapeutic interventions at the surface.

CO_2 laser is known as the most successful laser type to increase enamel's acid resistance. High absorption of this laser by hydroxyapatite radicals is the cause of this resistance [30]. Studies have shown that the wavelength of 9 to 11 μm are well absorbed by tooth hydroxyapatite which reduces the amount of carbonate and thus increases the acid resistance [31–34]. Also, the 10.6-μm wavelength of this laser has a low absorption coefficient and results in greater durability compared with wavelength of 9.6 μm. Therefore, it has more penetration into greater depths of the enamel and is suggested for prevention of caries [35]. This laser increases fluoride uptake [36] and in the presence of fluoride can convert hydroxyapatite into fluorapatite [37]. Therefore, in this study, CO_2 laser with wavelength of 10.6 μm was used for remineralization and treatment of WSLs, and the results showed that this laser is effective in improving the microhardness.

The findings of this research were similar to Poosti et al. [13], Chen et al. [38], and also Esteves-Oliveira et al. [39] regarding the positive effect of CO_2 laser in recovering the hardness of the demineralized enamel. Increased microhardness after laser irradiation can be caused by nanostructural changes

Chart 3 Comparison of the means microhardness of study groups in different depths



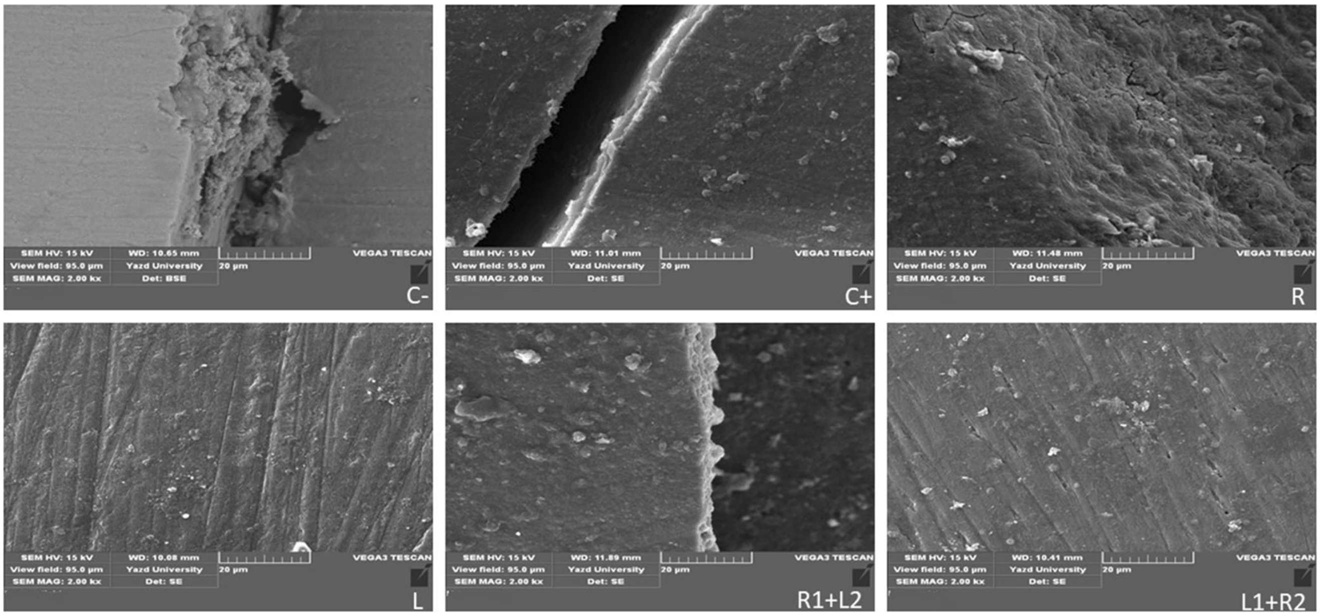


Fig. 5 Scanning electron microscope images prepared from the sample surface of each study group at × 2000 magnifications

such as increasing the size of crystals and recrystallization of porous enamel after increasing the surface temperature [39, 40].

In this study, the use of Remin Pro alone and in combination with laser was effective in increasing the microhardness of WSLs, so that in all groups that Remin Pro was used, significant difference with negative control group was observed. Microhardness improvement in the groups with combination therapy might be caused by nanostructural changes caused by the laser.

Most of the claimed properties for the paste are proved by various studies. Kamath U et al. found that the Remin Pro increases bleached enamel hardness [41]. Heravi et al. also reported that using Remin Pro in a 12-week period improves and reduces WSLs in patients after orthodontic treatment [42].

However, the microhardness mean of the group L1 + R2 at the surface and the depth of 50 μm was less than that of group R, but at the depths of 100 μm and 150 μm, the opposite was observed. In other words, probably the laser irradiation made effectiveness possible in the mentioned depths by facilitating Remin Pro penetration into the depths of the sample. Despite the higher microhardness of the group L1 + R2 compared with the R1 + L2 in all study depths, the mean difference was only significant at the depths of 100 and 150 μm. Possibly, facilitating penetration of Remin Pro by the laser has increased its effect in depth, while the presence of Remin Pro during laser irradiation has reduced its effect further. The microhardness of group L1 + R2 was more than that of group L in all depths. In other words, the laser in combination with Remin Pro had more effect that confirms the synergistic effect of laser and Remin Pro. The microhardness of group L1 + R2 was greater than that of group C+ in all depths, but the difference was only significant in depths of 100 and 150 μm. In other words, the effect of laser and Remin Pro in lower depths was equivalent to other therapeutic interventions (brushing and using mouthwash), but in the deeper depths that treatment interventions had lower effects, the effect of laser and Remin Pro were greater. The results showed that the combination therapy was more effective than individual therapy with laser CO_2 and Remin Pro, and it can also return significantly the microhardness of WSLs deeply. These results were matched with findings of Poosti et al., in that more effect of combination therapy and also the better effect of laser radiation prior to the application of Remin Pro. Tepper et al. [43], Schmidlin et al. [44], and Mohan et al. [45] also proved synergistic effect of CO_2 laser with fluoride.

In a number of studies [30, 36, 46], the mechanism of synergistic effect of laser and fluoride has been described as the reduction of enamel solubility. The use of fluoride before or after laser irradiation mentioned the cause of increased fluoride uptake and eventually decreased enamel solubility in acid. Critical pH for enamel dissolution is 5.5, but this amount decreases to 4.8 with laser irradiation which means enamel dissolution following laser irradiation requires far more concentration of acid [35, 47]. Laser irradiation causes more durability of fluoride ions but the mechanism of this durability has not yet been determined [48]. However in some studies, the synergistic effect has not been observed, thus further studies are required in this regard [32].

The microhardness of group R1 + L2 was higher than that of group L at the surface, but in other depths it was lower. This result may be caused because Remin Pro has prevented the laser from penetrating. The paste prevents laser-induced heat and thus the microstructural and chemical changes of enamel. This finding is consistent with Poosti et al.'s report in the use of APF gel before laser radiation [13].

This study shows that CO_2 laser irradiation before Remin Pro has better effect. This is probably due to the ability of laser to penetrate in to deeper enamel layers and reharden demineralized enamel. In general, it can be said that the laser irradiation before applying fluoride increased its uptake. In fact, laser causes the formation of microspaces in enamel that facilitates the fluoride activity [49]. Some studies have confirmed this hypothesis, for example, in the study of Hossain et al., laser irradiation before applying NaF increased the adhesion of fluoride to the lower layers of enamel and dentin [46].

The SEM images of the samples in different study groups showed the creation of cracks and fine scrapes in groups with laser irradiation. It seems that the possible cause of laser's ability to create cracks on WSLs compared with healthy enamel was the demineralized and weak surface of the lesions. Creating cracks and grooves by laser can entrap and deposit minerals in the tooth structure during a bacterial attack, and on the other hand, the aforementioned defects might act as a niche for the adhesion and the establishment of bacteria.

The SEM images of the treated surfaces with Remin Pro showed globular pattern of minerals. Covering the enamel surface with Remin Pro may create a protective layer against decay and demineralization. The findings of this research were similar to the study of Ahrari et al., who observed a relatively similar pattern in their study. They mentioned that the cause of this morphological pattern is calcium, phosphate, and fluoride inactivity at the surfaces that were under laser irradiation [50]. Wu et al. reported that CO_2 laser irradiation with 10.6 μm wavelength integrates the hexagonal crystals of the enamel surface and creates a surface with new recrystallization [51]. While in some studies, the need for integration of enamel crystals has not been approved for increasing the enamel resistance to demineralization [52–54]. Tepper et al. also found that laser irradiation can cause cracks in the enamel surface, and applying fluoride before laser irradiation can reduce crack formation [43]. In general, it can be concluded that microcracks, which are created on the enamel surface after laser irradiation, can be one of the causes of improved penetration of minerals in to the enamel's crystalline structure.

Conclusion

- The results of the present study showed that the use of CO_2 laser and Remin Pro significantly increased the microhardness of WSLs.
- This study confirmed the synergistic effect of laser and Remin Pro paste in the deep layers of enamel (150 μm), but in the other studied depths (0 μm, 50 μm, 100 μm) no significant synergistic effect was observed. The combination of CO_2 laser with Remin Pro, if laser is irradiated before applying the paste, is more effective in the deep layers of enamel than individual laser therapy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The ethics committee of the university approved the study (IR.SSU.REC.1396.188) before the sampling took place.

References

1. Årtun J, Brobakken BO (1986) Prevalence of carious white spots after orthodontic treatment with multibonded appliances. The European Journal of Orthodontics 8(4):229–234
2. Mann AB, Dickinson ME. Nanomechanics, chemistry and structure at the enamel surface. The teeth and their environment. 19: Karger Publishers; 2006. p. 105–31
3. Lone E, Utreja A, Singh S, Jena A (2015) Effect of multibracket orthodontic appliance on frequency and severity of enamel demineralization-a prospective study. Journal of Dental Specialities 3(1):36–39
4. Paula ABP, Fernandes AR, Coelho AS, Marto CM, Ferreira MM, Caramelo F, et. al.. Therapies for white spot lesions—a systematic review. Journal of Evidence Based Dental Practice 2017;17(1):23–38
5. Cochrane N, Cai F, Huq N, Burrow M, Reynolds E (2010) New approaches to enhanced remineralization of tooth enamel. J Dent Res 89(11):1187–1197
6. Sudjalim T, Woods M, Manton D (2006) Prevention of white spot lesions in orthodontic practice: a contemporary review. Aust Dent J 51(4):284–289
7. Øgaard B, Rolla G, Øgaard BJOMS, Clinical Aspects. Stuttgart T. Oral microbiological changes, long-term enamel alterations due to decalcification, and caries prophylactic aspects. 2001:123–42
8. Azrak B, Callaway A, Knözinger S, Willershausen B. Reduction of the pH-values of whole saliva after the intake of apple juice containing beverages in children and adults. Oral health & preventive dentistry. 2003;1(3)
9. Susan A-K, Forsberg C-M, de Jong EdJ, Angmar-Månsson B. A longitudinal laser fluorescence study of white spot lesions in orthodontic patients. Am J Orthod Dentofac Orthop 1998;113(6):595–602
10. Shungin D, Olsson AI, Persson M. Orthodontic treatment-related white spot lesions: a 14-year prospective quantitative follow-up,

including bonding material assessment American Journal of Orthodontics and Dentofacial Orthopedics. 2010;138(2):136. e1-. e8
11. Benjamin S, Roshni PS, Nainan MT (2012) Seal that heals. World J Dent 3(3):243–246
12. VOCO. Instructions for use of Remin Pro. 2010. Aug 1 Jan 6, 2012 [Available from: https://www.voco.dental/in/portaldata/1/resources/products/folders/gb/remin-pro-forte_fol_gb.pdf
13. Poosti M, Ahrari F, Moosavi H, Najjaran H (2014) The effect of fractional CO_2 laser irradiation on remineralization of enamel white spot lesions. Lasers Med Sci 29(4):1349–1355
14. Castellan CS, Luiz AC, Bezinelli LM, Lopes RM, Mendes FM, Eduardo CDP, et. al.. In vitro evaluation of enamel demineralization after Er: YAG and Nd: YAG laser irradiation on primary teeth. Photomed Laser Surg 2007;25(2):85–90
15. Pogrel M, Muff D, Marshall G (1993) Structural changes in dental enamel induced by high energy continuous wave carbon dioxide laser. Lasers Surg Med 13(1):89–96
16. Heravi F, Ahrari F, Mahdavi M, Basafa S (2014) Comparative evaluation of the effect of Er: YAG laser and low level laser irradiation combined with CPP-ACPF cream on treatment of enamel caries. J Clin Exp Dent 6(2):e121
17. Kumar V, Itthagarun A, King N (2008) The effect of casein phosphopeptide-amorphous calcium phosphate on remineralization of artificial caries-like lesions: an in vitro study. Aust Dent J 53(1): 34–40
18. Lata S, Varghese N, Varughese JM (2010) Remineralization potential of fluoride and amorphous calcium phosphate-casein phospho peptide on enamel lesions: an in vitro comparative evaluation. Journal of conservative dentistry: JCD 13(1):42
19. Derks A, Katsaros C, Frencken J, Van't Hof M, Kuijpers-Jagtman AJCr. Caries-inhibiting effect of preventive measures during orthodontic treatment with fixed appliances 2004;38(5):413–420
20. Heymann HO, Swift Jr EJ, Ritter AV. Sturdevant's art & science of operative dentistry-E-Book: Elsevier Health Sciences; 2014
21. Davidson C, Hoekstra I, Arends JJCr. Microhardness of sound, decalcified and etched tooth enamel related to the calcium content 1974;8(2):135–144
22. Kodaka T, Debari K, Yamada M, Kuroiwa MJCr. Correlation between microhardness and mineral content in sound human enamel 1992;26(2):139–141
23. González-Rodríguez A, de Dios López-González J, Del Castillo JdDL, Villalba-Moreno J. Comparison of effects of diode laser and CO_2 laser on human teeth and their usefulness in topical fluoridation. Lasers Med Sci 2011;26(3):317–324
24. Hsu J, Fox J, Wang Z, Powell G, Otsuka M, Higuchi W (1998) Combined effects of laser irradiation/solution fluoride ion on enamel demineralization. J Clin Laser Med Surg 16(2):93–105
25. Knoop F, Peters CG, Emerson WBJJoRotNBos. A sensitive pyramidal-diamond tool for indentation measurements 1939;23(1):39
26. Marsillac MdWSd, Delbem ACB, Vieira RdSJBJoOS. Effect of time in hardness test on artificially demineralized human dental enamel 2008:1507–1511
27. Purdell-Lewis DJ, Groeneveld A, Arends J (1976) Microhardness and densitometric measurements of the effect of 4% SnF2 solution on artificial white spot lesions. Caries Res 10(3):216–226
28. Paes Leme AF, Tabchoury CP, Zero DT, Cury JA (2003) Effect of fluoridated dentifrice and acidulated phosphate fluoride application on early artificial carious lesions. Am J Dent 16(2):91–95
29. Delbem AC, Cury JA (2002) Effect of application time of APF and NaF gels on microhardness and fluoride uptake of in vitro enamel caries. Am J Dent 15(3):169–172
30. Ana P, Bachmann L (2006) Zezell DJLp. Lasers effects on enamel for caries prevention 16(5):865
31. Steiner-Oliveira C, Rodrigues LK, Soares LE, Martin AA, Zezell DM, Nobre-Dos-Santos MJDmj. Chemical, morphological and thermal effects of 10.6-μm CO_2 laser on the inhibition of enamel demineralization. 2006;25(3):455–62
32. Seino PY, Freitas PM, Marques MM, de Souza Almeida FC, Botta SB, Moreira MS (2015) Influence of CO_2 (10.6 mum) and Nd:YAG laser irradiation on the prevention of enamel caries around orthodontic brackets. Lasers Med Sci 30(2):611–616
33. Souza-Gabriel A, Colucci V, Turssi C, Serra M, Corona S (2010) Microhardness and SEM after CO_2 laser irradiation or fluoride treatment in human and bovine enamel. Microsc Res Tech 73(11): 1030–1035
34. Fried D, Ragadio JN, Akrivou M, Featherstone JD, Murray MW, Dickenson KMJJobo. Dental hard tissue modification and removal using sealed transverse excited atmospheric-pressure lasers operating at lambda= 9.6 and 10.6 um. 2001;6(2):231–9
35. Fox J, Yu D, Otsuka M, Higuchi W, Wong J, Powell GJCr. Combined effects of laser irradiation and chemical inhibitors on the dissolution of dental enamel 1992;26(5):333–339
36. Tagomori S, Morioka T (1989) Combined effects of laser and fluoride on acid resistance of human dental enamel. Caries Res 23(4): 225–231
37. Meurman JH, Hemmerle J, Voegel JC, Rauhamaa-Makinen R, Luomanen M (1997) Transformation of hydroxyapatite to fluorapatite by irradiation with high-energy CO_2 laser. Caries Res 31(5):397–400
38. Chen C-C, Huang S-TJP, surgery l. The effects of lasers and fluoride on the acid resistance of decalcified human enamel 2009;27(3): 447–452
39. Esteves-Oliveira M, Pasaporti C, Heussen N, Eduardo C, Lampert F, Apel C (2011) Rehardening of acid-softened enamel and prevention of enamel softening through CO 2 laser irradiation. J Dent 39(6):414–421
40. Kawasaki K, Tanaka Y, Takagi OJAoob. Crystallographic analysis of demineralized human enamel treated by laser-irradiation or remineralization 2000;45(9):797–804
41. Kamath U, Sheth H, Mullur D, Soubhagya M (2013) The effect of Remin Pro® on bleached enamel hardness: an in-vitro study. Indian J Dent Res 24(6):690
42. Heravi F, Ahrari F, Tanbakuchi B (2018) Effectiveness of MI paste plus and Remin Pro on remineralization and color improvement of postorthodontic white spot lesions. Dental research journal 15(2):95
43. Tepper SA, Zehnder M, Pajarola GF, Schmidlin PR (2004) Increased fluoride uptake and acid resistance by CO_2 laser-irradiation through topically applied fluoride on human enamel in vitro. J Dent 32(8):635–641
44. Schmidlin PR, Döriga I, Lussi A, Roos M, Imfeld TJOh, dentistry p. CO 2 laser-irradiation through topically applied fluoride increases acid resistance of demineralised human enamel in vitro. 2007;5(3)
45. Mohan AG, Ebenezar AR, Ghani MF, Martina L, Narayanan A, Mony BJEjod. Surface and mineral changes of enamel with different remineralizing agents in conjunction with carbon-dioxide laser 2014;8(1):118
46. Hossain MM, Hossain M, Kimura Y, Kinoshita J, Yamada Y, Matsumoto K (2002) Acquired acid resistance of enamel and dentin by CO_2 laser irradiation with sodium fluoride solution. J Clin Laser Med Surg 20(2):77–82
47. Fox J, Yu D, Otsuka M, Higuchi W, Wong J, Powell GJJodr. Initial dissolution rate studies on dental enamel after CO_2 laser irradiation. 1992;71(7):1389–98

48. Delbem ACB, Cury J, Nakassima C, Gouveia V, Theodoro LHJJoclm, surgery. Effect of Er: YAG laser on CaF2 formation and its anti-cariogenic action on human enamel: an in vitro study. 2003;21(4):197–201
49. Oho T, Morioka TJCr. A possible mechanism of acquired acid resistance of human dental enamel by laser irradiation 1990;24(2): 86–92
50. Ahrari F, Mohammadipour HS, Hajimomenian L, Fallah-Rastegar A (2018) The effect of diode laser irradiation associated with photoabsorbing agents containing remineralizing materials on microhardness, morphology and chemical structure of early enamel caries. J Clin Exp Dent 10(10):e955–ee62
51. Wu CC, Roan RT, Chen JHJLiS, Medicine MTOJotASfL, Surgery. Sintering mechanism of the CaF2 on hydroxyapatite by a 10.6-lμm CO_2 laser. 2002;31(5):333–8
52. Hsu CY, Jordan TH, Dederich DN, Wefel JS (2001) Laser-matrix-fluoride effects on enamel demineralization. J Dent Res 80(9): 1797–1801
53. Kantorowitz Z, Featherstone JD, Fried D. Caries prevention by CO_2 laser treatment: dependency on the number of pulses used. Journal of the American Dental Association (1939). 1998;129(5):585–91
54. McCormack S, Fried D, Featherstone J, Glena R, Seka WJJodr. Scanning electron microscope observations of CO_2 laser effects on dental enamel. 1995;74(10):1702–8