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Effect of Er,Cr:YSGG Laser and Remin Pro on Microhardness of Enamel White Spot Lesions Induced by Orthodontic Treatment

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Abstract

Background and objectives: White spot lesions (WSLs) are one of problems caused by orthodontics treatment, resulting from enamel demineralization and leading to compromised esthetics and mechanical integrity. Recently, sub-ablative erbium laser irradiation and remineralizing agents like Remin Pro toothpaste (a hydroxyapatite-based remineralizing agent) have been proposed as potential approaches to enhance enamel resistance to demineralization. This in vitro study aimed to evaluate the effect of Er,Cr:YSGG laser irradiation and Remin Pro toothpaste on the microhardness of enamel white spot lesions following orthodontic treatment.

Materials and methods: Fifty sound extracted premolars were selected, and artificial white spot lesions were created using a standardized demineralization protocol. The samples were randomly allocated into experimental groups, including Er,Cr:YSGG laser irradiation, Remin Pro application, and a combination of both interventions. Laser irradiation was performed under sub-ablative conditions using an Er,Cr:YSGG laser (wavelength: 2780 nm) with predefined parameters for power, pulse mode, energy density, and water cooling. Enamel microhardness was assessed at different depths using a Vickers microhardness tester. Data were analyzed using SPSS (version 23) via two-way ANOVA and Tukey's tests at a 0.05 significance level.

Results: Statistically significant differences in enamel microhardness were observed among the experimental groups as well as across different enamel depths ($P < 0.05$), with the highest microhardness values recorded in the combined laser and Remin Pro group in 150 micrometers depth.

Conclusions: Within the limitations of this in vitro study, Er,Cr:YSGG laser irradiation, particularly when combined with Remin Pro toothpaste, may enhance the mechanical integrity of enamel white spot lesions and improve their resistance to demineralization.

Keywords: Er,Cr:YSGG laser, Remin Pro, White spot lesions, Microhardness, Orthodontics.

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1. Introduction

Although orthodontics treatments enhance facial aesthetic and teeth position, they associated with increased dental plaque accumulation, teeth demineralization and discoloration as a side effect (1,2). White spot lesions (WSLs) are one of problems caused by orthodontics treatment and refer to localized hypomineralization areas that limited to enamel and clinically visible as opaque white area. WSL has unpleasing appearance and might progress into dental carries which need restorative management (3,4). WSL prevalence varies between 23.4% to 96% and it has been increasing in recent years, particularly in patients undergoing orthodontic treatment (5,6). In some cases, salivary proteins may partially neutralized chalky appearance and remineralize the enamel surface (7). However, in orthodontics treatment process, these lesions progress rapidly and become irreversible, result in carious lesions (8). Various methods had been introduced to manage WSL, like topical fluorides, fluoridecontaining bonding materials, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP)-containing products, laser therapy, resin infiltration, and micro-abrasion (9).

In recent years, it has been shown that laser irradiation has remineralizing effect on tooth structure. It has been indicated that high power lasers, such as CO2, erbium lasers (erbium:yttrium-aluminium-garnet (Er:YAG), and erbium, chromium:yattrium-scandium-gallium-garnet (Er,Cr:YSGG) have beneficial effects in white spot lesion management (10). Erbium lasers are mainly applied for hard tissue ablation due to their high absorption in water and hydroxyapatite (10). However, under sub-ablative conditions, erbium laser irradiation can modify the enamel surface without tissue removal and improve resistance to demineralization and the effectiveness of remineralizing agents (11-13).

Recently, Remin Pro, a new remineralizing agent, was introduced to the market. This water-based cream contains calcium and phosphate in hydroxyapatite form, fluoride and xylitol. Eroded enamel fills with hydroxyapatite, dentin tubules seals with fluoride and xylitol have antibacterial effects. Remin pro has been suggested for management of enamel demineralization during orthodontics treatment and to prevent and control tooth hypersensitivity after bleaching after bleaching. It’s also preferred in patients with bovine proteins allergy, since lacking bovine proteins present in CPP-ACP (14,15).

This study aimed to evaluate the efficacy of the Er,Cr:YSGG laser and Remin Pro paste in the treatment of white spot lesions (WSLs) and to compare the effects of their combined application with those of each treatment used alone.

2. Materials and methods

Considering the significance level (α) of 5% and the power of a test (β) (80%) and according to the results of the previous similar study (16) and the standard deviation of enamel hardness equal to (SD) (3) and to achieve a difference of at least 3 units in the average hardness of two groups, the number of 10 samples in each group (50 samples) is required.

We used 50 recently extracted sound premolars as samples. Teeth with any decay, enamel cracks, and structural defects were excluded from the study. If the teeth are damaged during any step of the test or the microhardness cannot be measured, that sample will be removed. Each extracted tooth was cleaned of blood and soft tissue remnants, washed with normal and disinfected according to Centers for Disease Control and Prevention (CDC) guidelines by autoclave sterilization at 121°C and 15psi for 15 minutes (17). This protocol has been previously reported to provide effective disinfection of extracted teeth without causing a statically significant alteration in enamel microhardness. Following disinfection, samples were kept in normal saline solution until the test.

The samples were randomly divided into 5 groups of 10. Then they were placed in 10 ml of demineralizing solution, for 96 hours to create artificial enamel white spots (18). The demineralizing solution consists of 0.05 mol of lactic acid, 2.2 mmol of calcium chloride ($CaCl_2$), 2.2 mmol of sodium dihydrogen phosphate (NaH_2PO_4) and 0.2 ppm fluoride, the solution was mixed with 50% NaOH until its pH = 4.5 (19). The solution was changed daily. After this process, the samples were washed with distilled water, Then, all the surfaces of the teeth but C- group, were covered with two layers of nail polish, except for a 4 × 4 window in the center of the buccal surface as the treatment area. samples were divided into the following 4 groups:

1) Group C- (negative control group): Ten of the samples were buried in transparent acrylic to ensure the effect of the acid solution on the samples, and they were weared from the interproximal area, until half of the buried tooth remained in acrylic. The buccal region of the crown of the samples at the depths of zero, 50, 100 and 150 micrometers were examined by Knoop microhardness. Other samples were kept in artificial saliva until the end of the process.

2) Group C+ (positive control group): The samples in this group were not subjected to any treatment; in order to simulate the period of intraoral remineralization they were kept in artificial saliva for 90 days. In the last 14 days, they were exposed to fluoride mouthwash (Vi-One®, Vi-One Co., Iran) for 60 seconds, Merident toothpaste (Pars Darou Co., Tehran, Iran) was then applied to the enamel surface using a soft-bristle toothbrush (Patrix®, Iran) and left undisturbed for 5 minutes once a day. After each procedure, the samples were rinsed with distilled water and stored in artificial saliva until the next cycle.

3) Group L: Group L (laser group) consisted of 10 samples that were irradiated using an Er,Cr:YSGG laser (erbium, chromium: yttrium-scandium-gallium-garnet) (Waterlase®, Biolase Inc., Irvine, CA, USA) with a wavelength of 2780 nm. Laser irradiation was performed at an output power of 1.2 W, pulse repetition rate of 30 Hz, and an energy of 40 mJ per pulse, operating in long-pulse mode with a pulse duration of approximately 140 µs. The Er,Cr:YSGG laser was operated in long-pulse (H) mode to provide controlled sub-ablative energy delivery and reduce the risk of enamel ablation and microcrack formation compared to short-pulse modes (20). The laser was applied with a water spray of 50% and air spray of 10%. The laser tip was positioned perpendicular to the enamel surface at a distance of 6–8 mm, with a cross-sectional area of 0.8 mm^2, and irradiation was performed for 5 seconds. The calculated energy density was approximately 5 J/cm^2. Following laser irradiation, the samples were stored in artificial saliva for 90 days, to simulate intraoral remineralization conditions. In last 14 days, samples were exposed to fluoride mouthwash (Vi-One®, Vi-One Co., Iran) for 60 seconds, Merident toothpaste (Pars Darou Co., Tehran, Iran) was then applied to the enamel surface using a soft-bristle toothbrush (Patrix®, Iran) and left undisturbed for 5 minutes once a day. After each procedure, the samples were rinsed with distilled water and stored in artificial saliva until the next cycle.

4) Group R (Remin Pro paste): In this group, only the mentioned paste was applied on 10 teeth, so that the samples were placed in artificial saliva for 90 days and in order to simulate the period of intraoral remineralization, they were exposed to daily fluoride mouthwash containing 0.05% sodium fluoride (Vi-One®, Vi-One Co., Iran) for 60 seconds, then Remin Pro toothpaste (VOCO GmbH, Cuxhaven, Germany) was applied on a soft-bristle toothbrush (Patrix®, Iran) and left undisturbed for 5 minutes, once a day in last 14 days. After each Remin Pro application, the samples were rinsed with distilled water and stored in artificial saliva until the next cycle.

5) Group L+R (Er,Cr:YSGG Laser radiation + Remin Pro): In this group, 10 teeth were subjected to laser radiation. Then the samples were placed in artificial saliva for 90 days and in order to simulate the period of intraoral remineralization. In last 14 days, they were also exposed to daily fluoride mouthwash containing 0.05% sodium fluoride (Vi-One®, Vi-One Co., Iran) for 60 seconds, Remin Pro toothpaste (VOCO GmbH, Cuxhaven, Germany) was then applied to the enamel surface using a soft-bristle toothbrush (Patrix®, Iran) and left undisturbed for 5 minutes. After each procedure, the samples were rinsed with distilled water and stored in artificial saliva until the next cycle.

After the experiment, the samples were removed from the artificial saliva. Then, they were buried in clear acrylic, and from the interproximal area, they were abraded using a diamond disc until half of the tooth remained buried in acrylic. In the next step, due to the necessity of polishing the surface of the samples to perform the microhardness test firstly, they were polished serially with polishing disks (USA, Paul, St, ESPE, M3 (Soflex) from harder to softer under the cooling water flow. In the second step, using silicon carbide papers from 6000 hardness to the softest carbide paper mounted on rotating plates, the samples were polished again under the cooling water flow.

After polishing the samples, the hardness profile was measured with a Knoop indenter (China, SCTMC Z-1000MHV) at the depths of zero, 50, 100 and 150 micrometers from the external border of the enamel. The hardness of the samples was measured on the surface of the enamel and then at 50 micrometer intervals to avoid overlapping areas under hardness testing. To increase the accuracy of the measurement at each depth, this operation was done three times and then the average result was recorded.

After checking the microhardness of the samples by the Knoop hardness tester, the numbers obtained for each sample were recorded and entered into the computer for analysis.

Statistical analysis was done by statistical software (SPSS version 23) and two-way ANOVA tests and Tukey's multiple comparisons were used. Data were analyzed statistically at a significance level of 0.05.

3. Results

Data normality was confirmed using the Kolmogorov–Smirnov test ($P > 0.05$); therefore, parametric statistical analyses were performed. Analysis of variance (ANOVA) followed by post hoc Tukey tests was used for intergroup comparisons.

The mean enamel microhardness values (mean ± SD) of the experimental groups at enamel depths of 0, 50, 100, and 150 µm are presented in Tables 1–4. Overall analysis demonstrated statistically significant differences in enamel microhardness among the four groups at all evaluated depths ($P < 0.05$).

At 0 µm depth, statistically significant intergroup differences were observed ($P < 0.05$, Table 1). The L group exhibited the highest mean microhardness value, while the lowest value was observed in the C- group. Pairwise comparisons showed that the L+R group differed significantly from the R, C+, and C- groups; in all these comparisons, the mean microhardness of the L+R group was higher. However, the mean microhardness of the L+R group was lower than that of the L group (Figure 1).

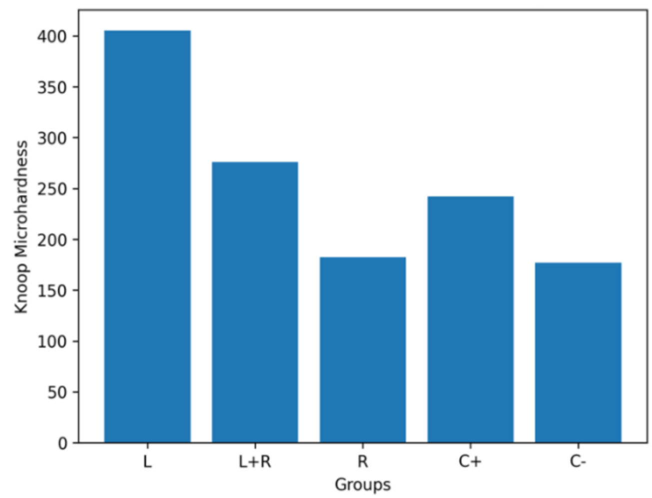

Figure 1. Comparison of enamel microhardness values among the experimental groups at 0 µm depth.

At 50 µm depth, statistically significant differences among the groups were also detected ($P < 0.05$, Table 1). The L group again demonstrated the highest mean microhardness, whereas the lowest value was recorded in the C- group. Pairwise comparisons indicated that the L+R group showed significantly higher microhardness values than the R, C+, and C- groups; in all these comparisons, the mean microhardness of the L+R group was higher, while it remained lower than that of the L group (Figure 2).

At 100 µm depth, intergroup differences in enamel microhardness remained statistically significant ($P < 0.05$, Table 1). The highest mean microhardness value was observed in the L group, and the lowest in the C- group. Pairwise comparisons revealed that the L+R group differed significantly from all groups except the L group; in all statistically significant comparisons, the mean microhardness of the L+R group was higher (Figure 3).

At 150 µm depth, statistically significant differences among the experimental groups were observed ($P < 0.05$, Table 1). At this depth, the L+R group exhibited the highest mean microhardness value, while the lowest value was observed in

Table 1. Mean ± SD enamel microhardness values of the experimental groups.

Group	Mean ± SD			
Depth	0 µm	50 µm	100 µm	150 µm
C-	177.02 ± 30.77	203/94 ± 33/90	226/12 ± 34/21	239/51 ± 27/72
C+	242.24 ± 48.17	237/06 ± 23/60	229/37 ± 23/09	214/01 ± 24/08
L	405.18 ± 70.18	406/87 ± 66/06	373/64 ± 41/56	358/86 ± 49/59
R	182.49 ± 24.13	226/48 ± 23/35	275/18 ± 200/9	294/66 ± 25/36
L+R	275/.97± 29.15	316/36 ± 23/72	362/56 ± 317/7	383/88 ± 27/35
P-value	0.0001	0.0001	0.0001	0.0001

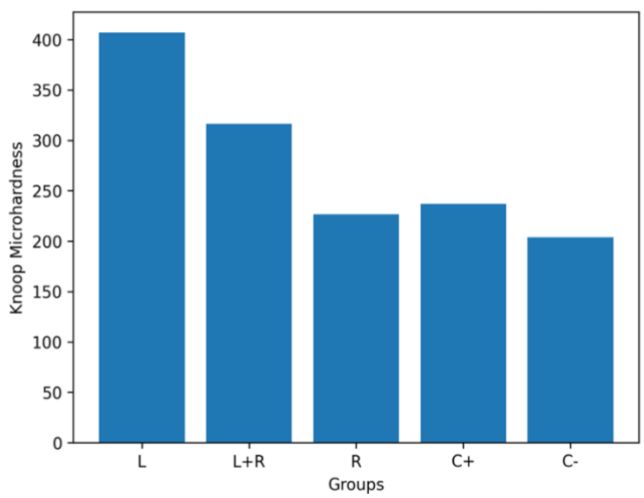

Figure 2. Comparison of enamel microhardness values among the experimental groups at 50 µm depth.

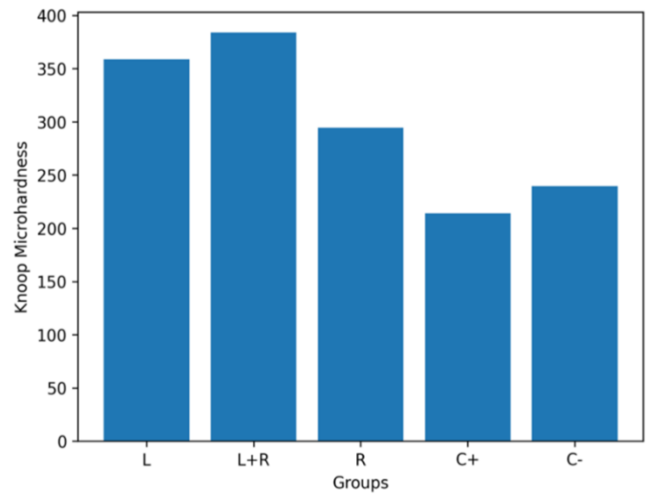

Figure 3. Comparison of enamel microhardness values among the experimental groups at 150 µm depth.

the C- group. Pairwise comparisons demonstrated that the L+R group showed statistically significant differences compared with all groups except the L group; in all these comparisons, the mean microhardness of the L+R group was higher. These findings are illustrated in Figure 4.

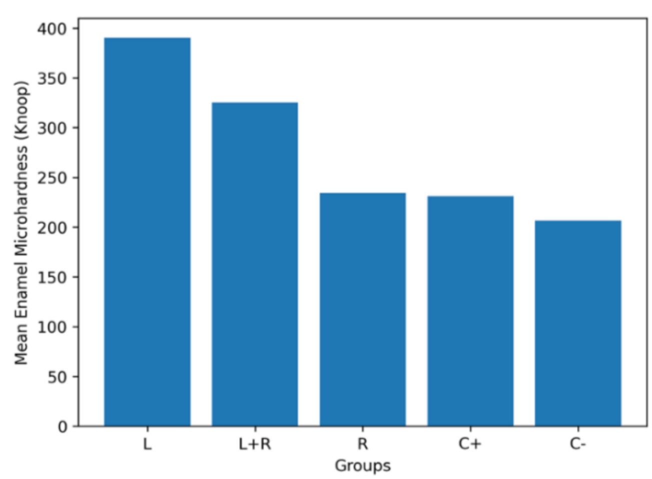

Figure 4. Overall comparison of enamel microhardness values across different depths and experimental groups.

4. Discussion

White spot lesions (WSLs) are one of the fixed orthodontic treatment side effects and represent subsurface enamel demineralization beneath an apparently intact surface layer, leading to compromised teeth strength and esthetics (3,4,8,21). Their high reported prevalence during orthodontic treatment indicates the need for evidence-based preventive and therapeutic strategies (5,6). In the present in vitro study, enamel microhardness was used as the primary outcome measure, as it is a validated indirect indicator of mineral content and mechanical integrity of enamel (8,22).
The results demonstrated that Er,Cr:YSGG laser irradiation alone produced the highest microhardness values at depths of

0, 50, and 100 µm. This finding is consistent with previous studies reporting that erbium lasers induce physicochemical alterations in enamel, including crystal melting and recrystallization, reduction of carbonate content, and decreased enamel permeability, ultimately increasing resistance to acid dissolution (10,11,23,24). Castellan et al. showed that erbium laser irradiation significantly reduced enamel demineralization, while Yassaei et al. reported enhanced microhardness of laser-treated white spot lesions, particularly in superficial enamel layers (23,24).

Remin Pro paste also produced a statistically significant increase in enamel microhardness (14,15). This effect can be attributed to its biomimetic composition, which includes hydroxyapatite, fluoride, and xylitol. Hydroxyapatite acts as a calcium and phosphate reservoir, helping mineral deposition, while fluoride enhances enamel resistance to acidic challenges by promoting fluorapatite (9,25,26). These findings are in agreement with last studies demonstrating improved enamel hardness following the application of Remin Pro and similar hydroxyapatite-based remineralizing agents (26-28).

Nevertheless, Remin Pro paste alone was less effective than laser irradiation at all evaluated depths. This observation supports previous evidence indicating that topical remineralizing agents primarily affect the enamel surface and may have limited capacity to change deeper enamel structure (6,29).

The absence of a synergistic effect between laser irradiation and Remin Pro paste in superficial enamel layers (0, 50, and 100 µm) may be explained by laser-induced surface sealing, which can reduce enamel porosity and limit ion penetration (30). However, at a depth of 150 µm, the combined laser and Remin Pro treatment showed the highest microhardness values, indicating a depth-dependent synergistic effect. Similar findings have been reported in studies evaluating combined laser irradiation and remineralizing agent protocols, suggesting enhanced subsurface remineralization over time (2,30).

The positive control group, exposed only to conventional fluoride toothpaste, demonstrated limited remineralization, reinforcing evidence that routine use of fluoride may be insufficient for reversing established white spot lesions (5,6).

5. Limitations

Artificial saliva was used in this study; however, using natural saliva - or an artificial substitute that more closely replicates the enzymes and minerals found in natural saliva - could increase the strength of the study. The present study was performed in vitro, and further studies should be conducted in a clinical setting to investigate the effectiveness of treatment methods in these patients.

6. Conclusion

The results of this study showed that application of Er,CR:YSGG laser result in WSLs remineralization and Remin Pro paste also improves enamel hardness. We did not find a synergic effect for laser and paste at depths 0, 50, 100 micrometers, but the results showed their synergic effect at 150 micrometers. We did not observe a synergistic effect between the laser and the paste at depths of 0, 50, and 100 µm; however, a synergistic effect was observed at a depth of 150 µm. According to the findings of this study Er,Cr:YSGG laser and Remin Pro paste can be effective in the treatment of WSLs and have a synergic effect at deeper levels.

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None.

Ethics
This experimental study was approved by the ethics committee of Shahid Sadoughi University of Medical Sciences with IR.SSU.REC.1399.197 ethical code.

Using artificial intelligence (AI)
No artificial intelligence–based tools were employed in any stage of this research (i.e., study design, analysis, manuscript preparation, etc.).

Author contributions
Mohammad Hossein Toodehzaeim: Conceptualization, Supervision, Project administration, Writing - Review & editing
Hanieh Validad: Investigation, Writing - Original draft, Writing - Review & editing
Elahe Rafiei: Data curation, Supervision, Methodology
Alireza Haerian: Investigation, Methodology
Reza Molla: Project administration, Investigation
Mohammad Safaei: Investigation, Project administration, Data curation

Conflict of interest
The authors declare no conflict of interest.

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