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Effect of Diode Laser and Fluoride Varnish on Microhardness of enamel: An In Vitro Study

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Abstract

Introduction: White spots are a common complication of orthodontic treatment. Several prevention methods such as fluoride therapy and laser irradiation have been proposed, but there is still no conclusive evidence. Therefore, this study aimed to evaluate the combined application of diode laser and fluoride varnish in preventing white spots.

Methods: Thirty-nine sound human premolars were extracted for orthodontic purposes and randomly divided into three groups of thirteen teeth each (C: Control, FV: fluoride varnish (5% sodium fluoride), and FV + L: fluoride varnish + 980 nm diode laser (2 W)). The freshly extracted teeth were disinfected in a 0.5% chloramine T solution and stored in saline at room temperature. Each tooth was coated with nail polish, leaving a 2 × 3 mm^2 window on the midbuccal. Surface treatments were applied, and primary Vickers microhardness was evaluated in the gingival sections. Then, the samples were immersed in alternating demineralizing (6 hours/day) and remineralizing (18 hours/day) solutions and incubated at 37 °C for 9 days to produce artificial caries. Afterward, the samples were immersed in a remineralizing solution for one day. Finally, secondary microhardness was measured. The data were analyzed using one-way ANOVA.

Results: The primary microhardness was higher in groups C and FV than in group FV + L. The lowest secondary microhardness was recorded in group C, and there was no significant difference between the other two groups. The change in microhardness was greater in group C, while no difference was observed between the FV and FV + L groups.

Conclusion: Within the limitations of this study, the combined application of laser irradiation and fluoride varnish may not improve white spot lesion (WSL) prevention compared to the use of fluoride varnish alone.

Keywords: White spot; Fluoride; Diode laser; Microhardness.

Introduction

Smile improvement is a major motivator for patients seeking orthodontic treatment, leading to an increase in the number of patients seeking treatment.[1] Unfortunately, white spot lesions (WSLs) are one of the most common complications of fixed orthodontic treatment, particularly affecting the esthetics of anterior teeth.[2,3] Despite recent advances in materials and techniques, the WSL has been reported in nearly 50% of patients undergoing fixed orthodontic treatment.[4] On average, one-third of orthodontic patients experience at least one WSL during treatment.[5] A 46% incidence of the WSL has been reported in patients undergoing fixed orthodontic treatment at 12 months after bracket bonding.[6] WSLs are clinically visible demineralization of the enamel surface.[7] Difficulty in maintaining oral hygiene and the disruption of saliva's self-cleansing ability during lip and cheek movements make patients with fixed orthodontic appliances susceptible to plaque accumulation and therefore WSL development.[8] Regular brushing with fluoride toothpaste is paramount to prevent WSLs. Additionally, fluoride mouthwashes can significantly reduce WSL formation.[9] Toothpastes consist of monofluorophosphate, sodium fluoride, stannous fluoride, amine fluoride, or combinations of these substances. Among them, stannous fluorides prevent plaque formation by absorbing bacteria on the enamel surface.[9] Oral hygiene instruction, professional supervision, and encouraging patients to maintain optimal oral hygiene are also effective factors in preventing WSL.[4]

Previous researches have demonstrated the synergistic effect of combining anti-caries agents such as fluoride and laser irradiation in preventing dental caries. The

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mechanism is attributed to an increased ability of enamel to retain fluoride and an increased conversion rate of hydroxyapatite to fluorapatite after laser irradiation.[10,11] In this regard, a study indicated the effectiveness of the combination of diode laser and sodium fluoride in absorbing fluoride on tooth enamel.[12] However, another research has demonstrated a significant reduction in enamel acid solubility and prevention of caries development in vitro. Lasers can cause acid resistance, preventing the formation and development of dental caries. The combined use of fluoride and laser provided the most promising results in preventing tooth caries.[13]

Although there is some evidence regarding the effects of multi-stage laser irradiation on the demineralization and prevention of WSL,[14] no studies have investigated the effects of multi-stage laser irradiation for the prevention of WSL in permanent teeth. Previous studies performed on successive teeth have used an agent other than fluoride[15,16] or single-stage laser irradiation.[17,18] Therefore, this study aimed to evaluate the effectiveness of simultaneous three-stage 980 nm diode laser irradiation, with a power of 2 watts and fluoride varnish-containing pigment in preventing WSL.

Methods

The experiment was performed on 39 sound human premolars that were extracted for orthodontic purposes. Informed consent was obtained from all subjects or legal guardians if the subjects were under 18 years old to allow the use of the extracted teeth. Teeth with caries, lesions, and hypoplastic enamel were excluded from the study. The samples were disinfected by soaking in 0.5% chloramine T for a week, and then stored in saline solution at room temperature.[19] The remaining tissues and calculus were removed by scaling. The teeth were cut 1 mm apically to the cementoenamel junction (CEJ). The specimens were mounted in self-curing acrylic resin in a way that the surfaces of the samples were higher than the acrylic resins and the specimens did not merge into the resin (Figure 1). The teeth surfaces were then smoothed and polished under water using 800, 1000, and 2000 grit sandpapers in one direction for 15, 30, and 60 seconds, respectively. Each tooth was coated with nail polish, leaving a 2×3 mm^2 window on the midbuccal. Vickers microhardness was measured using the Vickers hardness tester (Bareiss, Germany) at a force of 100 g for 10 seconds. An average of three indentations, 50 µm apart, were recorded in the gingival part of the window. The samples were then divided into three groups, with 13 samples randomly assigned to each group using the RAND function of Excel software. One tooth in each group was prepared for examination of the tooth surface topography using a scanning electron microscope (SEM).

Figure 1. A Sample Mounted in Acrylic Resin

- Group 1 (control, C): No treatment was applied.
- Group 2 (fluoride varnish, FV): Samples were placed in 5% sodium fluoride (NaF) varnish (Clear cavity varnish, Vericom, Korea) for 4 minutes and then stored in saline for 6 hours. The area was then thoroughly cleaned with a toothbrush and distilled water.
- Group 3 (fluoride varnish + laser, FV + L): After the application of fluoride varnish similar to group 2, the teeth were irradiated using a diode laser (Simpler, Doctor Smile, Italy) with a wavelength of 980 nm, a diameter of 8 mm, an energy density of 120 J/cm^2 per 30 seconds (360 J/cm^2 in total), and 2 W of power at a one-mm distance. Irradiation was performed three times for 30 seconds each time with one-minute intervals. After 4 minutes, the samples were placed in saline for 6 hours. The area was then thoroughly cleaned with a toothbrush and distilled water (14).

Developing Artificial Caries Lesions

All teeth underwent a process of demineralization and remineralization to form artificial carious lesions. The samples were placed in Ten-Cat acid with a pH = 5, containing 2.20 mmol of calcium, 2.20 mmol of phosphate, and 50 mmol of buffer (acetic acid/potassium acetate) for 6 hours daily. This was done to simulate the demineralization process of caries formation. After that, the samples were placed in a mineralization solution with a concentration of 20 mmol for 18 hours at 37 °C to initiate the remineralization phase of caries development.[20] The remineralization solution used was supersaturated with calcium phosphate (calcium 1.5 mmol/L, phosphate 0.9 mmol/L), potassium chloride (150 mmol/L) and cacodyl buffer (20 mmol/L) at a pH of 7. This solution had a saturation level close to the salivary mineral apatite, similar to Ten Cate and Dujisters.[21] This cycle was repeated for 9 days, with the solution being changed every other day. Throughout the 9-day cycle, the samples were kept in an incubator at 37 °C (INB400, Memmert, Germany) to simulate the oral environment. Afterwards, the samples were immersed in the remineralization solution for one day and the secondary microhardness was measured using the Vickers hardness tester at a force of 100 g for 10 seconds. An average of three indentations, 50 µm apart, were recorded in the gingival part of the window.

Enamel Surface Topography

For SEM analysis, the enamel samples were cleaned with acetone, dehydrated with ethanol (at a gradient concentration of 70%, 80%, 90%, and 100% for 20 minutes), and left to dry at room temperature for 24 hours. The samples were then sputter-coated with gold. The surface topography of enamel was evaluated under a Hitachi S4160 FE-SEM machine.

Statistical Analysis

The data were presented as mean and standard deviation (SD). The results were analyzed using one-way ANOVA and paired *t* test. Post hoc tests, including Tukey HSD and Tamhane, were used. Statistical significance was considered at $P < 0.05$.

Results

Microhardness

The greatest primary microhardness was measured in the group C (471.36 ± 36.10) followed by the varnish fluoride group (469.26 ± 27.51) and the FV + L group (440.59 ± 38.15). The greatest secondary microhardness was recorded in the FV group (317.43 ± 43.60) followed by the FV + L group (308.62 ± 27.60) and the group C (302.58 ± 34.98). The group C showed the greatest change in microhardness (302.58 ± 34.98), while the FV + L group showed the least change (131.97 ± 36.56) (Table 1 and Figure 2).

Both primary and secondary microhardness showed significant differences between the groups ($P < 0.001$). Pairwise comparisons of primary and secondary microhardness between the groups were made using Tukey HSD or Tamhane post hoc tests, depending on the variance. Primary microhardness showed no significant difference between the group C and the group FV. The primary microhardness of both the control and fluoride varnish groups was significantly higher than that of the FV + L group (Table 2).

The secondary microhardness of the control group was significantly lower than that of the fluoride varnish and FV + L groups ($P < 0.001$). There was not a significant difference between the FV group and the FV + L group (Table 3).

Table 1. Primary and Secondary Microhardness Mean and Its Changes in Control, Fluoride Varnish, and Fluoride Varnish + Laser Groups

Microhardness	Group	N	Mean	SD
Primary	C	13	471.54	36.10
	FV	13	469.26	27.51
	FV + L	13	440.59	38.15
Secondary	C	13	168.96	36.24
	FV	13	317.92	43.60
	FV + L	13	308.62	27.60
Changes	C	13	302.58	34.98
	FV	13	151.33	39.79
	FV + L	13	131.97	36.56

C: control; FV: fluoride varnish; FV + L: fluoride varnish + laser; N: number; SD: standard deviation.

Changes in Microhardness

Changes in microhardness were evaluated using a paired t-test. The primary microhardness was decreased by 302.59 ± 34.98, 151.33 ± 39.80, and 131.97 ± 36.56 compared to the secondary microhardness in the C, FV, and FV + L groups, respectively (Table 4, Figure 3). The microhardness change was significantly different between the groups. The control group showed the greatest reduction in microhardness among all groups ($P < 0.001$). There was no significant difference in the microhardness change between the FV and FV + L groups (Table 5).

Enamel Surface Topography

There was no significant difference in the enamel surface topography between the control group and the experimental groups (Figure 4).

Discussion

White spots are a common complication of orthodontic treatment, and various methods have been proposed to address this issue, including fluoride compounds, remineralizing agents, tooth bleaching, composite resin, indirect restoration, and laser treatment.[22] In recent years, diode lasers have become increasingly popular in dentistry, and it has been claimed that it may improve the resistance of teeth to caries regardless of the use of fluoride agents.[15] The exact mechanism of how lasers play a role in fluoride absorption into tooth enamel is still unknown, but two mechanisms have been proposed. First, the thermal effects of the laser increase fluoride uptake, and second, the microcracks created by the laser treatment trap fluoride within the tooth.[23,24] The laser also reduces the content of carbonate ions, making tooth enamel less soluble and increasing the content of fluoride ions near the enamel. Additionally, the laser alters the organic enamel, making it less susceptible to cavities. However,

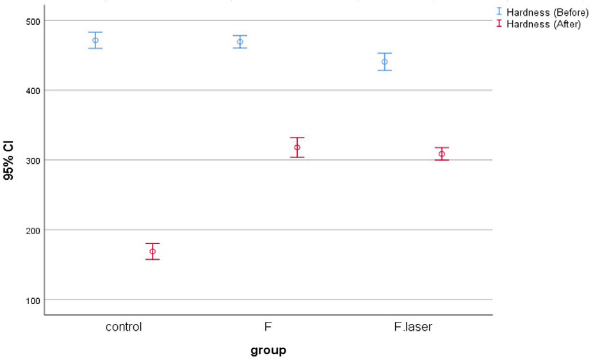

Figure 2. Mean Microhardness in the Study Groups

Table 2. Pairwise Comparison of Primary Microhardness Between Groups According to Tukey HSD Post Hoc Test

Groups		Mean Difference	*P* Value
I	II		
C	FV	2.28	0.95
C	FV + L	30.49	< 0.001*
FV	FV + L	28.66	0.001*

C: control. FV: fluoride varnish. FV + L: fluoride varnish + laser.
* Significant according to *P* value less than 0.05.

Table 3. Pairwise Comparison of Secondary Microhardness Between Groups According to Tamhane Post Hoc Test

Groups		Mean Difference	*P* Value
I	II		
C	FV	-148.97	< 0.001*
C	FV + L	-139.66	< 0.001*
FV	FV + L	9.30	0.492

C: control. FV: fluoride varnish. FV + L: fluoride varnish + laser.
* Significant according to *P* value less than 0.05.

since lasers require expensive equipment,[25] it is important to investigate their effects on dental changes. Although there is evidence supporting the use of multistage laser irradiation for preventing demineralization and WSLs,[14] there is a lack of studies investigating the effects of multiple laser irradiations on permanent teeth. Either the sample used was a deciduous tooth, the intermediate material was not fluoride,[15,16] or the irradiation was not performed in multiple stages.[13] Therefore, due to the lack of sufficient evidence, this study used a three-step laser diode irradiation with a wavelength of 980 nm, a power of 2 W, an irradiation interval of 30 seconds, and a break of one minute. Fluoride varnish was also used to extend our experimental groups.

The surface layer of the tooth plays a significant role in tooth decay, so it is important to assess changes in this layer.[15] Microhardness testing is suitable for non-uniform and crack-prone materials with fine microstructures, such as enamel. Microhardness testing is an effective method for analysing the impact of materials on enamel remineralization and is considered non-invasive and easy to perform.[26] Similar to the studies by Rafiei et al[22] and Mahmoudzadeh et al,[2] the Vickers index was used in the present study to measure microhardness. This method is more accurate when measuring the location of the diamond pyramid effect because the indentation dimensions are measured in two directions compared to the Knop test, which is measured in only one direction.[26]

The results of this study showed significant differences in primary microhardness levels between the study groups. The FV + L group had the lowest primary microhardness, while the control and fluoride varnish groups showed no significant difference at the initial stage. The overall average values of primary microhardness of the studied groups were higher than the values obtained in the study of Alqahtani et al.[27] According to del Pilar Gutiérrez-Salazar and Reyes-Gasga,[28] microhardness ranges from 327 to 397 when measured parallel to the occlusal surface. Differences in sample preparation techniques can account for this variation.

Table 4. Microhardness Change (Primary Microhardness – Secondary Microhardness) in Study Groups

Groups	Mean Microhardness Change	SD	*P* Value
C	302.59	34.98	< 0.001*
FV	151.33	39.80	< 0.001*
FV + L	131.97	36.56	< 0.001*

C: control. FV: fluoride varnish. FV + L: fluoride varnish + laser.
* Significant according to *P* value less than 0.05.

Table 5. Pairwise Comparison of Microhardness Change Between Groups According to Tukey HSD Post Hoc Test

Groups		Microhardness change difference	P value
I	II		
C	FV	151.26	< 0.001*
C	FV + L	170.62	< 0.001*
FV	FV + L	19.36	0.060

C: control. FV: fluoride varnish. FV + L: fluoride varnish + laser.
* Significant according to *P* value less than 0.05.

Secondary microhardness was also significantly different in the study groups. The control group had the lowest value. The FV + L and fluoride varnish groups were statistically similar. Consistent with the present results, the in vitro studies by de Almeida Baldini Cardoso et al[29] and Mohd Said et al[30] showed that enamel microhardness significantly improved after the use of 5% fluoride varnish. Similarly, this study found that adding a laser to fluoride may not improve microhardness as the amounts of microhardness change were not statistically significant between the FV + L and fluoride varnish groups. In contrast, Hashemikamangar et al reported that microhardness values within NaF, NaF–blue laser, and NaF-Er:YAG laser were recovered after the demineralization cycle to the level of the baseline, and this increase was higher in groups with laser irradiation. They also pointed out that laser alone has no meaningful effect on microhardness.[31] The photothermal interaction of laser and hydroxyapatite was mentioned as one of the possible mechanisms for obtaining this outcome. Differences in laser settings including power and mode of irradiation can account for dissimilar findings. Nakagaki et al[32] and Alqahtani et al[33] also reported a statistical effect of the combination of laser and fluoride compared to fluoride on microhardness. Differences in materials, methods, tooth type (animal vs human, deciduous vs permanent teeth), laser types and parameters, active ingredients of fluoride varnish and duration of use, and microhardness measurement methods (Vickers vs nope) can also account for the different results in various studies.

In the same year, Hashemikamangar et al evaluated

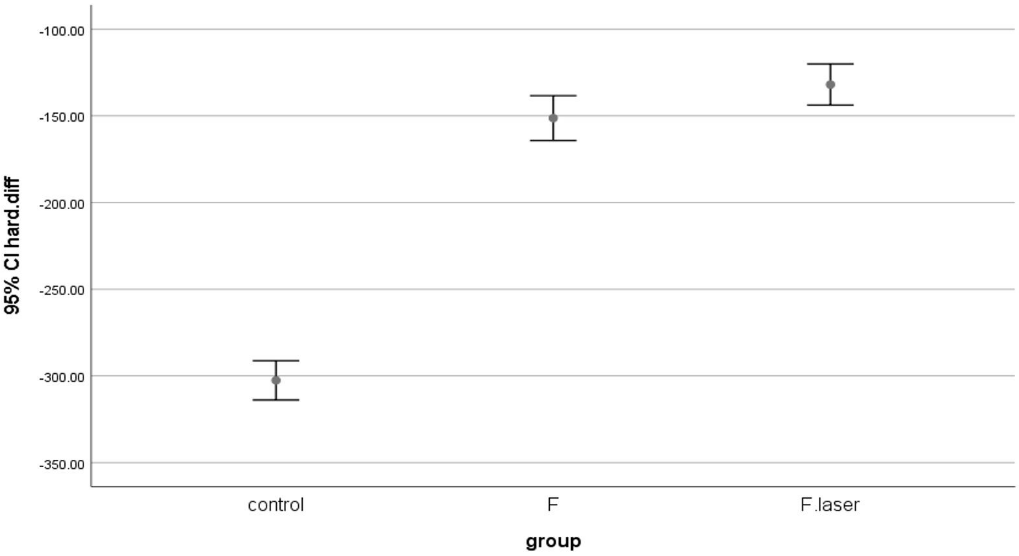

Figure 3. The Percentage of Microhardness Change (Primary Microhardness – Secondary Microhardness) in Study Groups

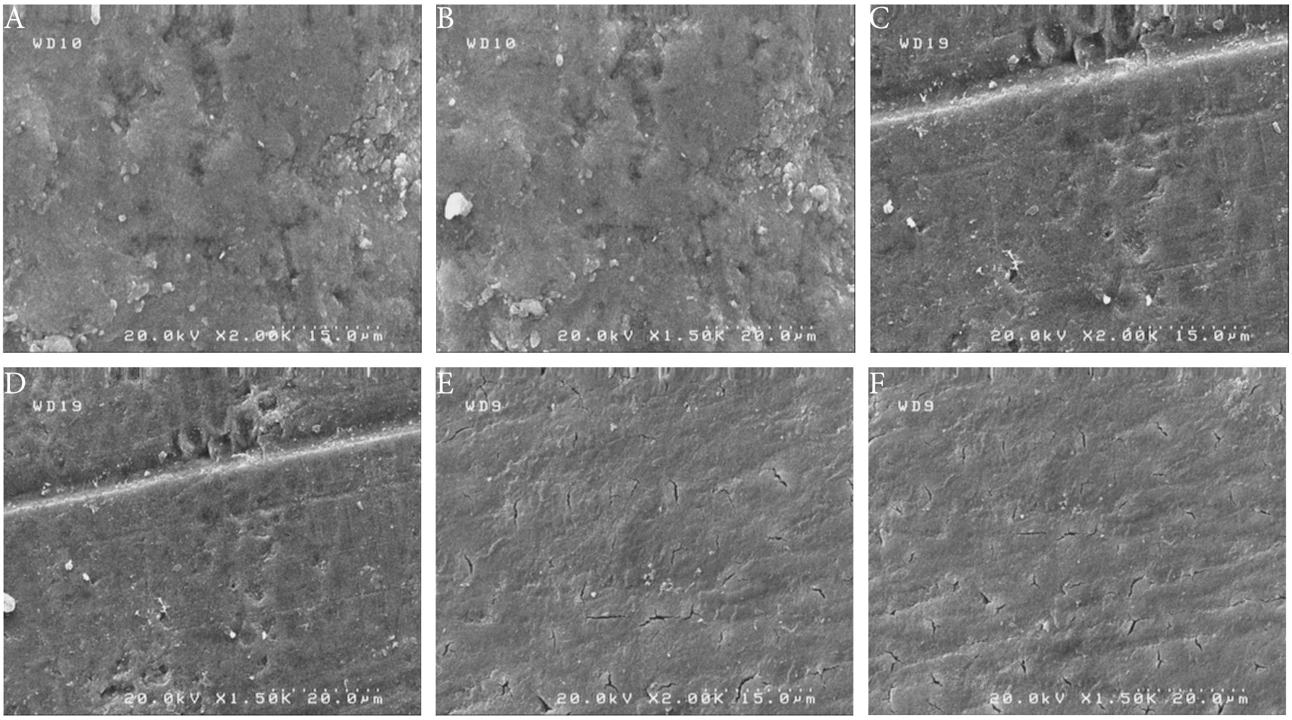

Figure 4. The Enamel Surface Topography Using a Scanning Electron Microscope. A: control group (×1500). B: control group (×2000). C: fluoride varnish group (×1500). D: fluoride varnish group (×2000). E: fluoride varnish+laser group (×1500). F: fluoride varnish+laser group (×2000)

the synergistic effect of photodynamic therapy and local fluoride on the microhardness of demineralized enamel and found that there is no cumulative effect between these two approaches and fluoride varnish can increase microhardness with or without photodynamic therapy by photosensitive agents, a finding which is in line with our results.[34] It was also noted that the highest decrease in enamel microhardness was in groups mediated by photodynamic therapy alone. According to a study by Mahdi and Hussein concerning the effect of Er,Cr:YSGG laser irradiation with or without fluoride on the enamel surface, samples treated by fluoride only showed an insignificant difference with ones treated by a combination of laser and fluoride in enamel microhardness.[35] Although they used different lasers and carried out their experiment on deciduous enamel, their results again confirm our findings. Laser induction of chemical and crystallographic alterations in dental mineralized tissues was noted as a possible factor in the empowered resistance of dental enamel.

According to Gouda et al, the sequence of surface treatments can have a pivotal effect on the final characteristic of the enamel superficial layer.[36] As they mentioned, enamel microhardness in groups with fluoride gel application after laser irradiation was significantly higher than samples with the laser alone, fluoride alone, or laser irradiation after fluoride gel application. The treatment sequence in our samples was in a way that the laser succeeded fluoride application, so it is similar to these findings. It can be estimated that the application of laser before fluoride application induces a thermal action that changes the enamel microstructure to better retain fluoride. According to Ulusoy et al, the synergistic effect of laser and fluoride application cannot be concluded as they found no significant difference in calcium and phosphate content of primary and permanent sound enamel samples treated whether by the laser alone or laser and fluoride combination.[37] The sequence of surface treatments in their research was similar to ours, but the laser type was Er,Cr:YSGG.

The importance of surface treatment sequence was also highlighted by Alshamrani et al. According to their findings, the Er,Cr:YSGG laser and fluoride + laser groups showed a statistically significant decrease in microhardness values between baseline and post-erosion measurements; however, the fluoride and laser + fluoride groups showed a significant increase in microhardness values compared to the baseline.[38] Abdulhussein and Al Haidar also confirmed the efficacy of laser application before using fluoride on the enamel of permanent teeth as their group modified by both laser and fluoride sowed significantly higher microhardness after demineralization.[39] These researches and also the research of Ana et al. seem to properly justify our findings as they noted an increase in the formation of calcium fluoride-like material on the enamel surface after exposing the tooth to Er,Cr:YSGG laser irradiation prior to fluoride application.[13]

On the other hand, Alshamrani noted that the application of laser after treatment by fluoride gel can significantly increase microhardness, which opposes our results and can be due to the enamel type, primary teeth, and also different fluoride gel concentration.[40]

The most similar research to our experiment was the one by Chokhachi Zadeh Moghadam et al which evaluated the effects of diode laser and fluoride on the prevention of enamel demineralization in primary teeth. They exposed the laser on enamel samples which were treated by fluoride varnish before that, similar to the sequence of our settings. They concluded that both fluoride and fluoride with a diode laser can effectively increase enamel microhardness and they do not differ significantly from each other, which is exactly the same as our outcomes.[14]

As we move through the literature, we can observe different and sometimes opposite findings about the synergistic effect of fluoride and laser combination with other types of lasers. For example, in a study conducted by Seino et al, no synergistic effect of the co2 laser and fluoride was observed,[41] while Poosti et al demonstrated the synergistic effect of acid phosphate (APF) and CO_2 laser.[42] Thus, future studies comparing the effectiveness of the fluoride-laser combination and the APF-laser combination are suggested.

In the current study, the surface topography of tooth enamel was not significantly different between the fluoride varnish and FV + L groups compared with the control group (Figure 4). In contrast, Lopes et al. demonstrated chemical changes in the enamel surface roughness in response to laser irradiation. This may be explained by differences in laser parameters such as power, energy density, and wavelength.[43]

This study met some limitations. First, measured surface microhardness can only assess remineralization. The transverse microradiography method is recommended for further studies. This method is the gold standard in evaluating mineral distribution in subsurface enamel lesions. This method uses a light microscope and a camera to photograph the enamel surface. Image analysis software then calculates the extent of mineral loss.[44] Another limitation of the present study was the variation in primary microhardness. It also would be better to expand the experimental groups to have broad coverage of different laser and fluoride application settings.

Further studies evaluating the influence of different laser parameters on microhardness are recommended. In addition to microhardness measurements, calcium and phosphorus ions measurement is required. Evaluation of the effectiveness of the combination of fluoride and laser in preventing WSL and treating these lesions in animal experiments and subsequent clinical trials is also proposed.

Conclusion

Within the limitations of this study, laser irradiation in addition to fluoride varnish may not improve WSL prevention compared to the use of fluoride varnish alone.

Authors' Contribution

Conceptualization: Sepideh Arab.
Data curation: Hanieh Najjaran, Mohammad Javad Kharazifard
Formal analysis: Mohammad Javad Kharazifard.
Investigation: Hanieh Najjaran, Nasim Chiniforush.
Methodology: Sepideh Arab, Sara Valizadeh, Nasim Chiniforush.
Project administration: Fatemeh Safari, Nasim Chiniforush.
Supervision: Sepideh Arab, Sara Valizadeh.
Writing–original draft: Fatemeh Safari.
Writing–review & editing: Sepideh Arab, Fatemeh Safari, Mohammad Ali Keshvad.

Competing Interests

The authors declare that they have no competing interests.

Ethical Approval

This study was approved by the Ethics Committee of Tehran

University of Medical Sciences (reference number: IR.TUMS.DENTISTRY.REC.1400.123). Informed consent was obtained from all subjects or legal guardians if the subjects were under 18 years old to allow the use of the extracted teeth.

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