Original Article

Comparative Evaluation of the Effect of Diode Laser Irradiation, Fluoride Varnish, and Casein Phosphopeptide-Calcium Amorphous Phosphate on Demineralized Enamel Microhardness

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Abstract

Introduction: Laser diode irradiation, the use of fluoride varnish, and casein phosphopeptide-calcium amorphous phosphate (CCP-ACP) paste have been suggested as effective methods to increase the enamel resistance to decay. The present study aimed to investigate the effect of fluoride varnish, CPP-ACP paste, and diode laser radiation on the microhardness of demineralized enamel. **Methods:** In this experimental study, 78 extracted human premolar teeth were selected and sectioned into two buccal and lingual halves. The samples were immersed in demineralization solution, and primary micro-hardness was measured. Thereafter, the samples were randomly assigned to six groups: control group, 5% fluoride varnish, CPP-ACP paste, diode laser (2W, 940nm) for 40 sec, laser combined with fluoride varnish, and CPP-ACP paste in combination with laser. The microhardness of the samples was measured again after different treatments. Data were analyzed in SPPS software (version 23) using repeated measures analysis of variance (RM ANOVA) ($P<0.05$). **Results:** There was no significant difference in the microhardness values among different groups ($P<0.05$). The comparison of the mean microhardness between before and after treatment for each group revealed a significant difference in the enamel microhardness only in the CPP-ACP paste ($P=0.003$) and diode laser ($P=0.009$) groups. **Conclusion:** As evidenced by the results of this study, the use of fluoride varnish, CPP-ACP paste, and diode laser radiation did not increase the microhardness and remineralization of tooth enamel. Moreover, laser diode irradiation with the parameters used in this study does not enhance the remineralization effect of fluoride varnish and CPP-ACP paste.

Keywords: CPP-ACP, Diode laser, Enamel microhardness, Enamel remineralization, Fluoride varnish

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Introduction

The enamel structure protects the tooth against external factors; however, this structure might undergo changes or sustain irreversible damages caused by factors such as the acidic environment produced due to bacterial activity (1). The acid produced by bacteria spreads over the tooth surface and dissolves carbonated hydroxyapatite mineral contents, resulting in tooth demineralization. Nonetheless, the demineralization process is reversible, and if the pH becomes neutral and adequate amounts of calcium and phosphate ions are available in the environment, the dissolved apatite crystals can form again (2). Enamel surface lesions should be identified as early as possible so that the enamel will have the opportunity to remineralize and be repaired.

One of the aims of modern dentistry is non-invasive treatment of non-cavitated carious lesions to prevent cavitation and preserve the integrity of sound enamel (3).

Some studies reported that drinking liquids such as fresh milk and mineral water might slightly recover the demineralized enamel surface (4). A wide variety of professional and home-used products are available for this purpose in different forms, too (5).

Fluoride has widely been confirmed as a remineralizing agent, which reacts with calcium and phosphate ions on the surface or superficial layers of enamel in the presence of oral fluids, replacing carbonated hydroxyapatite with fluorapatite (6).

The use of fluoride varnish is the standard technique for the application of fluoride in dental offices. Its popularity lies in its ease of use, safety, and acceptance by patients. Fluoride varnish contains high concentrations of fluoride and might remain on the tooth surface for several hours after its application and retain the capacity to release fluoride (7). Therefore, it is expected that pediatric dentists use fluoride varnish as a component of oral hygiene services in children aged 2 years and older at six month intervals (8). The possible concerns over the safety of fluoride varnishes are primarily related to the systemic effects resulting from the long-term use of fluoride, including an increased risk of enamel fluorosis and renal toxicity, apart from its acute local effects, such as contact allergy in the oral mucosa (9).

Novel products, including bioactive glass and casein-phosphopeptide-amorphous calcium phosphate (CPP-ACP), have been introduced to simultaneously decrease the risk of fluorosis and preserve the remineralization capacity (10). An ideal remineralization system should contain calcium and phosphate with bioavailability capacity, in addition to fluoride, in order to induce favorable surface remineralization (11). Casein phosphopeptide (CPP) is the predominant protein in cow milk, containing a cluster sequence of –Ser(P)-Ser(P)-Ser(P)-Glu-Glu amino acids. It is tasteless, has low antigenicity and can be purified in the form of CPP-ACP nano-complex (12). The CPP provides a high concentration of calcium and phosphate ions, along with fluoride ions on the tooth surface, by binding to the pellicle and plaque, preventing demineralization and increasing remineralization. Previous studies have assessed how incorporating CPP-ACP into toothpaste affects erosive lesions. Some studies have noticed the preventive effect of CPP-ACP on erosion, while some others have not reported such an effect (13).

Despite the cariostatic potential of fluoride, it cannot completely eliminate caries; therefore, some non-fluoride remineralizing agents have been tested and advocated. A novel method for caries prevention is the application of high-power lasers, such as CO2, Er: YAG, and Er, Cr: YSGG. They can modify hydroxyapatite and solubility and increase resistance against enamel demoralization. Clinicians have expressed considerable concern over the expenses and availability of high-energy lasers, apart from the serious damage they inflict on gingival tissues or the dental pulp. To overcome this concern, low-level lasers (Diodes with 660-980nm) were considered for enamel remineralization (14). The hydroxyapatite of the dental structure absorbs a low level of a wavelength of 809-960, while the rest is transferred as heat on the enamel surface and its adjacent structures (15).

Significant morphologic changes, including liquefaction, crystallization, crack formation, and porosity, have been observed on the enamel surface after the irradiation of Nd: YAG, diode (808 and 980nm), and Er, Cr: YSGG laser beams (16). The Nd: YAG and diode laser have been successfully used to evaluate the strength and increase in enamel hardness (17). Diode laser delivers a beam close to the infrared wavelength (970 nm) with parameters close to those of the Nd: YAG laser beams; nonetheless, it has a wider field of application and is more readily available (18). Among all types of lasers, the diode laser has unique characteristics, including a lower cost compared to other lasers, small size and easier use in the oral cavity because of the optic fibers (19). Although previous studies have established the effect of Nd: YAG laser on increasing the microhardness of enamel; however, studies have yielded contradictory results regarding the use of diode laser (17,19).

It has been established that microhardness is a useful test with high sensitivity that can be used for surface lesions since it can identify the early stages of demineralization (20). A decrease in the microhardness of tooth hard structures might increase its dissolution and destruction, dentin permeability, and problems during the repair process. Moreover, an increase in enamel surfaces microhardness increases its resistance to demineralization (21). The majority of studies focused on the effects of diode laser alone and some others on fluoride agents; accordingly, we considered the effects of simultaneous use of these preventive measures on the microhardness of demineralized enamel. The present study aimed to determine the optimal technique for the remineralization of white lesions.

Materials and Methods

After the approval of the study by the Ethics Committee of Zanjan University of Medical Sciences, 78 intact extracted human premolar teeth were collected from private dental offices and clinics within two months based on the inclusion and exclusion criteria. The teeth with evidence of fluorosis, enamel defects, and decalcification were excluded. The teeth were first cleaned with a surgical knife and then with a toothbrush. The teeth were immersed in 0.5% chloramine solution at room temperature until the time of the experiment (22).

The tooth crowns were removed at the cementoenamel junction (CEJ) with diamond disks (DFS, Softy, Longlife, Germany) under water spray. Thereafter, the samples were sectioned into buccal and lingual halves using a Mecatome machine (Pressi, France) under water cooling. The samples were evaluated under a stereomicroscope (Olympus, Shinjuku, Tokyo, Japan) under ×40 magnification to ensure the absence of any defects on the tooth surface structure, cracks, or caries. Following that, the samples were subjected to a pH-cycling procedure at 37ºC for 14 days, in which they were dynamically demineralized similar to that in the oral environment. The samples were immersed in a demineralizing solution (pH=4.6) for 8 h, followed by immersion in artificial saliva for 1 h and a remineralizing solution (pH=7) for 15 h each day (21).

The demineralizing and remineralizing solutions were replaced every day. These solutions were prepared daily in the laboratory. The demineralizing solution consisted of 0.05 mM $CaCl_2$, 2.2 mM NaH_2PO_4, and 50 mM acetic acid, and the remineralizing solution consisted of 20 mM HEPES, 1.5 mM Ca^{2+} ($CaCl_2$), 0.9 mM phosphate (KH_2PO_4), and 1 ppm fluoride (NaF) (22). Artificial saliva with a controlled pH of 6.75 (Nickceram Rzai Corp, Isfahan, Iran) was synthesized two days before the pH-cycling procedure.

The samples were mounted in self-cured acrylic resin blocks (Acropars, Kaveh, Tehran, Iran) measuring 2×2×1 cm with a 4×4-mm window on the buccal or lingual surface of the sample exposed. Vickers test was used to determine the initial (baseline) microhardness of each sample using a microhardness tester (Zwick/Roell, Germany) with a pyramidal diamond indenter exerting a 50-gr (500-gr) for 10 sec (23). The means were calculated after three measurements in different locations of each sample. Finally, the samples were randomly assigned to six groups (n=13 in each group) according to the treatment protocol (Figure 1).

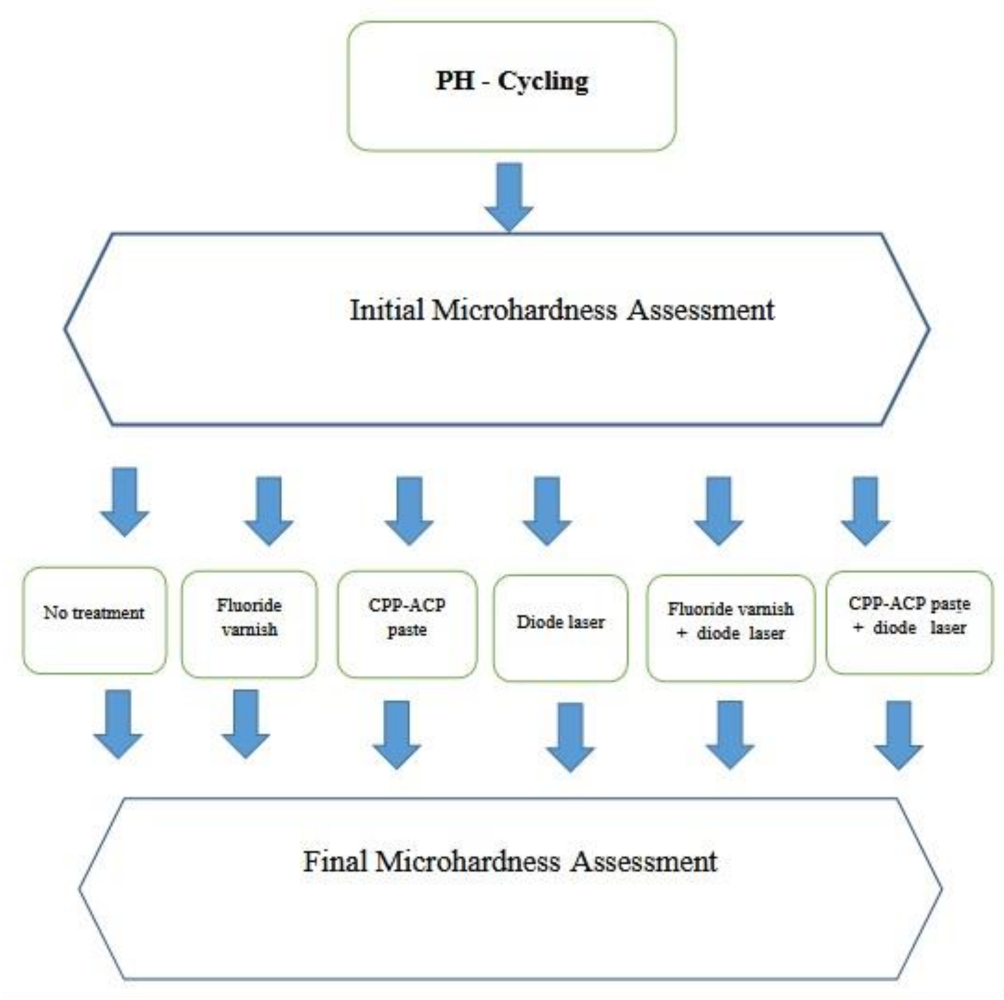

Figure 1. Schematic flowchart of the study protocol

Group 1 (control): Specimens received no treatment.

Group 2 (Fluoride varnish): 5% sodium fluoride varnish (DuraShield®, Sultan Corp, USA) was applied to the dry surface of the tooth using a microbrush and was left on the surface of the samples for 1 min according to the manufacturer's instructions. The teeth coated with fluoride varnish were immersed in the artificial saliva in separate containers for 24 h. After 24 h of contact with the tooth surface, the fluoride varnish was wiped off from the tooth surface with a piece of sterile gauze and a powered toothbrush (Oral-B Ez-5622, USA).

Group 3 (CPP-ACP paste): The CPP-ACP paste (GC Tooth Mousse, GC Corp., Japan) was applied to the tooth using a microbrush and was left on the surface of the samples for 5 min according to the manufacturer's instructions and then removed by rinsing with distilled water and stored in the artificial saliva.

Group 4 (diode laser): The samples were irradiated twice with diode laser beams (BIOLASE, ezLase 940, San Clemente, CA, USA) at a wavelength of 940 nm, an output power of 2 W, and an energy level of 80 J in the continuous wave mode. The duration of each radiation was 20 sec, with an interval of 60 sec (15, 23). The laser beams were delivered with a probe measuring 300µm in diameter, without any contact with the tooth surface at a distance of 10 mm from the surface using circular movements from the center outwards. The specimens were then stored in the artificial saliva.

Group 5 (fluoride varnish in combination with diode laser): The fluoride varnish was applied to the sample surface for one min similar to that in group 2. Subsequently, the diode laser beams were applied over the fluoride varnish immediately after the application of fluoride varnish on the tooth surface. The laser beam overcast the fluoride varnish and touched the samples at a standard distance of 1 mm. After laser irradiation, the fluoride varnish remained on the enamel surface and the samples were immersed in the artificial saliva in separate containers for 24 h. Fluoride varnish was then wiped off with a piece of sterile gauze and a powered toothbrush (Oral-B Ez-5622, USA), and the specimens were stored in the artificial saliva.

Group 6 (CPP-ACP paste combined with diode laser): The CPP-ACP paste was applied for 5 min similar to that in group 3 and removed by rinsing with distilled water. Thereafter, the diode laser beams were applied similar to that in group 4, and the specimens were stored in the artificial saliva. The samples were stored in artificial saliva until the re-measurements. After two days, the microhardness test was repeated on their surfaces. The means and standard deviations of the baseline and final microhardness, as well as the changes in microhardness, were determined in each group. Data were analyzed in SPSS software (version 23). Mean and standard deviation were reported for the microhardness before and after the intervention. The normal distribution of the data was confirmed using the Kolmogorov-Simonov test; therefore, ANOVA was used. Mean comparisons in each group before and after the intervention were performed by paired t-test. The level of significance was set at 0.05.

Results

Table I presents the means and standard deviations of demineralized enamel microhardness in groups before and after different preventive treatments. In this study, all samples had an initial microhardness value of about 291-331.5 Kg/mm2. Therefore, there were no statistically significant differences among the initial microhardness values of the groups prior to any treatment (P=0.276). Furthermore, the ANOVA test demonstrated no significant differences in enamel microhardness between the groups after the intervention (P=0.758). The lowest microhardness was recorded in the CPP-ACP group (272.2 Kg/mm^2), with the highest in the fluoride varnish group (294.9 Kg/mm^2) after the treatments.

The result obtained from post hoc analysis revealed that there were significant differences between initial and final microhardness assessments in control (P=0.023), CPP-ACP (P=0.003), and diode laser (P=0.009) groups. The means of microhardness values decreased from 317.7 to 272.2 Kg/mm2 and from 331.4 to 285.6 Kg/mm2 after the application of CCP-ACP paste and following diode laser beams, respectively (Table I). Combined fluoride-laser and CPP-ACP paste-laser did not increase the microhardness of demineralized enamel significantly. Figure 2 illustrates the microhardness changes before and after the intervention among the intervention groups**.**

Table I. Mean, standard deviation (SD), and interquartile range (IQR) of microhardness (Kg/mm^2) before and after intervention, as well as its change among the groups

	Before	After	Paired Differences	
Groups	Mean±SD	Mean±SD	Mean±SD	P-value*
Control	331.5±51.1	291.5±45.5	-39.98±55.12	0.023
Fluoride varnish	294.8±61.4	294.9±36.0	0.12±54.52	0.994
CPP-ACP paste	317.7±51.1	272.2±56.0	-45.53±45.12	0.003
Diode laser	331.4±32.0	285.6±54.1	-45.82±53.17	0.009
Fluoride varnish in combination with diode laser	291.2±65.8	293.7±47.7	2.56±42.72	0.833
CPP-ACP paste combined with diode laser	308.9±63.5	276.4±42.0	-32.54±72.67	0.132

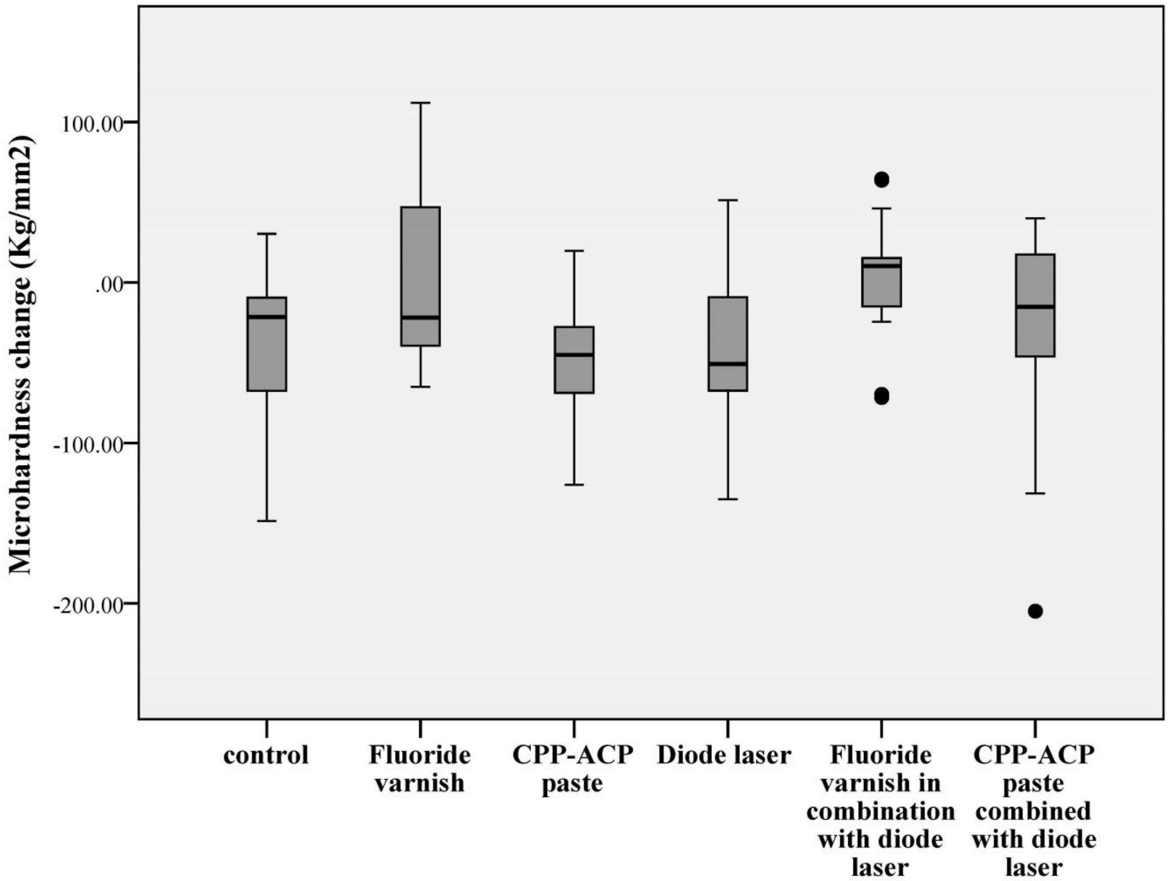

Figure 2. The microhardness changes (Kg/mm2) before and after the intervention among the intervention groups

Discussion

The prevention of dental caries is more important than its treatment (24), and many researchers have currently considered non-invasive techniques for the treatment of carious lesions (25). The remineralization of white spots and incipient caries prevents or delays the progression of caries and cavitation (26). Considering the relationship between the mineral content and microhardness of tooth enamel, the present study evaluated the effect of diode lasers, fluoride varnish, and CCP-ACP paste on the microhardness of demineralized enamel.

The results of the present study demonstrated no significant difference in the microhardness of demineralized enamel among the groups after treatment with different techniques. Some studies reported no significant difference among the groups in microhardness after the application of different techniques (19, 27). Nevertheless, others reported significant differences in enamel microhardness among the different groups after the application of CO_2 laser, CCP-ACP paste, and a combination of these two (22).

On the contrary, De Sant'Anna et al. indicated that diode laser beams (810 nm, 30 W) protected the enamel of deciduous teeth against acid attacks during the caries process with the use of a light-absorbing agent containing 2% sodium fluoride (28). A change in laser parameters, including the wavelength, power, pulse frequency, and duration of irradiation, might have resulted in various outcomes in different studies (29). Awooda et al. (ref?) measured the depth of caries-like lesions microscopically and demonstrated that the effective wavelengths were shorter, as in the case of 532 nm and 671 nm, while the other two wavelengths, as in 675 nm and 810 nm, revealed discrepancies regarding the depth of the lesion when compared to the control group.

From spectroscopy and absorption coefficient of enamel to the laser light, diode laser with a wavelength of 532 nm should give more acid resistance than the 671nm wavelength since it is located near the high absorption of laser light in the enamel tissue (30). Diode lasers are absorbed weakly in the enamel, and the near-infrared wavelength (810 nm) of diode laser does not seem effective; similar results were obtained by Kato et al. (31). In the present study, we used a diode laser at 2 W since higher power settings may produce pulpal damage clinically. Suleiman et al. irradiated the teeth for 30 sec with the diode laser at power settings of 1W, 2W, or 3W. They concluded that an increase in the pulp chamber temperature with the laser used at 1-2W was below the critical temperature increase of 5.5°C thought to produce irreversible pulpal damage. However, a power setting of 3W induced a pulp chamber temperature above this threshold (16°C); therefore, caution is advised in using this setting (32). Similar to the current study, some studies also used a diode laser with a power of 2W (15, 23).

The scanning electron microscope (SEM) evaluations by Ahrari et al. suggested that cracks and abrasions are produced on the enamel surface, and since white lesions have a weak and demineralized surface, the effect of laser beams on the induction of these cracks might increase, compared to the intact enamel (29). Therefore, since the samples in that study were subjected to a pH-cycling that resulted in enamel surface demineralization and the applied laser beams were stronger (2W) than those used by Ahrari et al. (ref?) (500 mW), a decrease was expected in microhardness due to diode laser irradiation.

In the present study, the highest microhardness was recorded with the sodium fluoride varnish, as well as a combination of varnish and diode laser; however, the differences between these two groups and the other groups were not significant. In this context, Moghaddam et al. reported no significant difference between fluoride varnish and a combination of diode laser and fluoride varnish in the loss of enamel surface microhardness (23).

Although the anti-cariogenic effects of fluoride-containing products have been well established (33), there are contradictory reports on the anti-cariogenic properties of laser irradiation in combination with fluoride (2, 19, 34). Some researchers believe that laser beams increase the fluoride content of enamel by enhancing the binding of fluoride to the tooth structure (35). Some others have hypothesized that the thermal effects of laser beams produce cracks in the enamel. The entrapment of minerals and fluoride ions in these cracks increases the resistance of the laser-irradiated surface to caries attack (36). In addition, some other researchers, such as Ahrari et al. and Bahrololumi et al., reported that although sodium fluoride varnish and its combination with diode laser beams increase the enamel microhardness, diode laser irradiation does not enhance the remineralizing effect of fluoride varnish (19, 29).

In the present study, CCP-ACP paste was used alone and in combination with a laser to treat demineralized enamel lesions. Some researchers have reported that the casein phosphate in the CCP-ACP paste stabilizes calcium and phosphate ions, facilitating the formation of calcium-phosphate nano-complexes on the tooth surface. Therefore, these compounds serve as a mineral matrix for remineralization. In the presence of casein phospholipids, the insoluble calcium phosphate becomes soluble; therefore, amorphous calcium phosphate forms on the tooth surface, serving as a reservoir for calcium and phosphate ions. This helps calcium and phosphate ions to deeply penetrate these lesions through the porous layer on the white lesions, resulting in the remineralization of enamel crystals. Moreover, this compound can rapidly transform into hydroxyapatite and precipitate on the tooth surface (37, 38).

In the present study, the use of CCP-ACP paste alone significantly decreased the microhardness of demineralized enamel, and the diode laser did not boost the effect of CCP-ACP paste, indicating that the use of this paste did not affect the demineralization of white lesions. Previous studies have used high-power lasers, such as CO_2, Nd: YAG, or Er: YAG, to increase the resistance of enamel to acid attacks (23, 39, 40). Some of these studies have indicated that the irradiation of tooth surface with CO_2 laser changes the calcium-to-phosphate ratio and decreases the carbonate-to-phosphate ratio (37). In addition, laser beams increase the recrystallization of the enamel structure, increasing its resistance to acid attacks (32). However, there is a dearth of studies on the effect of diode laser beams on the tooth structure, especially its microhardness (15, 17, 19, 29, 40).

In the present study, a low-power (2 W) diode laser was used for 40 sec. The results pointed out that laser irradiation alone did not decrease the microhardness of demineralized enamel significantly. Furthermore, a combination of laser beams and CCP-ACP paste decreased microhardness, and a combination of laser beams and fluoride varnish slightly increased microhardness. Ahrari et al. reported that although the application of diode laser beams (810 nm, 550 mW, 90 s) alone was not effective in increasing the microhardness of demineralized enamel, it increased enamel microhardness when it was combined with fluoride varnish and CCP-ACPF paste (29).

Salehzadeh Esfahani et al. reported that one-time application of CCP-ACP paste decreased the microhardness of demineralized enamel; however, one month after the application of CCP-ACP paste, microhardness increased significantly (26). Other studies have also reported the lowest microhardness values in the CCP-ACP group after its one-time application once when they used different preventive measures on demineralized enamel (22, 27). Nevertheless, in a study by Ahrari et al, (ref?) the microhardness of white lesions increased after the application of MI Paste Plus alone and in combination with laser beams. The MI Paste Plus contains 900 ppm of fluoride, in addition to CCP-ACP, which might explain the difference in the results of the stated study.

Previous studies have pointed out that the addition of fluoride to CCP-ACP increases the demineralization potential of enamel lesions, as compared to pure CCP-ACP or sodium fluoride alone. In addition, a lack of the effect of CCP-ACP paste might be related to its 5-min application according to the manufacturer's instructions. As illustrated in some studies, microhardness increased significantly after the use of CCP-ACP past for a month (26, 41). On the other hand, in the present study, after a 5-min application of CCP-ACP paste on the enamel, the paste was rinsed and removed from the tooth surface, which might have removed all the paste remnants from the tooth surface. Therefore, the duration of treatment was too short to bring about remineralization benefits. In the oral environment, an increase in the salivary flow due to food stimulants in association with soft tissue movements possibly removed the paste from the surfaces. Since the paste was applied according to the manufacturer's instructions, the clinical conditions are correctly reflected.

Oliveira et al. used quantitative light fluorescence (QLF) and reported that the remineralization potential of 1.1% sodium fluoride toothpaste was higher than that of 10% CCP-ACP. They believed that one of the reasons for the lower efficacy of CCP-ACP paste was the lack of biofilm in the *in vitro* study. In the absence of biofilms, there is no ideal environment for the CCP-ACP paste to create a super-saturated state of calcium and phosphate ions. Under such conditions, it is very unlikely for ACP to separate from carbon; therefore, ACP does not precipitate; moreover, the growth of crystals and remineralization do not take place (6).

It is suggested that future studies evaluate the effect of diode laser with other parameters, other lasers with different parameters, as well as CCP-ACP paste and fluoride varnish with longer application times, on the microhardness and remineralization of white lesions.

Conclusion

1. There were no significant differences in the microhardness of demineralized enamel among the groups after treatment with different techniques.
2. None of the preventive measures used in the present study resulted in a significant increase in the microhardness of demineralized enamel.
3. The use of CCP-ACP paste alone for 5 min significantly decreased the microhardness of demineralized enamel, and it seems that this duration is inadequate for the remineralization of white lesions.

4. The irradiation of diode laser beams with the parameters mentioned in the present study did not boost the effect of CCP-ACP paste and fluoride varnish.

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Conflicts of Interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article

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