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Dentinal Tubule Occlusion Using Diode Laser and Nano-Hydroxyapatite: An In-vitro SEM Analysis

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Abstract

Objective(s): This research aimed to evaluate the effect of diode laser and Remin Pro (containing nano-hydroxyapatite), both individually and in combination, on the occlusion of dentinal tubules. **Methods:** 45 extracted human premolar teeth were sectioned to create a 3×8 mm rectangle in the outer third of the cervical coronal dentin. The samples were then divided into five groups: negative control (with a smear layer), positive control (without a smear layer), diode laser (980 nm), Remin Pro, and combination of both. Dentinal tubule occlusion was evaluated using SEM at 2000x. Statistical analysis was performed using one-wayANOVA and LSD test at $p<0.05$. **Results:** The positive control exhibited the highest average percentage of open dentin tubules at 10.33%, while the combination treatment group showed the lowest percentage at 2.02%. When comparing the treatment methods to the positive control, all three treatments demonstrated statistically significant results ($P = 0.000$, 0.017, and 0.000). However, no significant statistical differences were found when the three treatment groups were compared to one another ($P= 0.279$, 0.935, and 0.733). **Conclusion:** All three treatment methods—Diode laser, Remin Pro, and their combination—effectively occluded dentinal tubules; however, none demonstrated superiority over the others.

Keywords: Dentin; Diode Laser; Dentin Hypersensitivity

Introduction

The loss of enamel due to abrasion, attrition, and erosion, along with the loss of cementum and periodontal disease, exposes dentin to the oral environment, leading to an increase in dentin hypersensitivity [1, 2]. This sensitivity is characterized by brief, sharp pain triggered by thermal, chemical (osmotic), and tactile stimuli[3]. Studies have reported the prevalence of dentin hypersensitivity to range from 4% to 73%, with higher rates observed in patients with periodontal disease [3]. The buccal surface, particularly in the premolar and canine regions, is usually the most affected area, followed by the molar regions [4]. Several theories have been proposed to explain the increase in dentin hypersensitivity, with Brännström's hydrodynamic theory being one of the most widely accepted mechanisms. This theory suggests that hypersensitivity is due to the inward or outward movement of fluid within the dentinal tubules and the mechanical displacement of nerve endings at the pulp-dentin junction. The number and diameter of open dentinal tubules determine the level of dentin sensitivity [5, 6].
To address dentin hypersensitivity, various treatments have been proposed. These include dietary recommendations, proper brushing techniques, occlusal adjustments, the use of adhesive systems, adhesive restorations, and agents such as potassium ions, oxalates, and sodium fluoride [7, 8]. Additionally, lasers, both low-power and high-power, have been investigated for their effectiveness in treating increased dentin sensitivity. When used correctly, lasers can seal dentinal tubules through a process known as "melting and resolidification," without causing damage to the pulp or cracking[9]. Diode lasers, with wavelengths of 780, 810, and 900 nm, are commonly used as low-power lasers for this purpose. A clinical study by Mobadder et al. in 2019 found that diode lasers are safe and effective for the long-term reduction of dentin sensitivity [10].
Remin Pro (VOCO, Germany) is a commercial product intended for topical application to alleviate dental sensitivity. Its formulation includes purified water, nano-hydroxyapatite (which contains calcium and phosphate), xylitol, and 1450 ppm fluoride. Nano-hydroxyapatite adheres to the tooth surface, helping to block the dentinal tubules. Xylitol is a sugar substitute known for its anti-cariogenic properties, as it cannot be converted into lactic acid by cariogenic bacteria, thereby allowing tooth remineralization. Additionally, fluoride also has anti-cariogenic effects and aids in the remineralization process. This product is free from milk proteins, making it suitable for individuals with milk protein allergies [11, 12].
Due to the inconsistent results observed in studies examining dentinal tubule occlusion with diode lasers and various desensitizing agents, and noting that Remin Pro has not been previously tested in conjunction with diode lasers, this study aimed to investigate the effectiveness of dentinal tubule occlusion using diode lasers and Remin Pro, both individually and in combination.

Methods

The current in vitro study was approved by the Ethics committee of Qazvin University of Medical Sciences (IR.QUMS..REC.1400.428). Forty-five human premolar teeth [13] that were extracted due to orthodontic treatment or periodontal issues were collected, ensuring that ethical considerations were observed. All teeth were free of caries and stored in a 0.1% thymol solution to prevent microbial growth until the study began. Initially, the dental samples were mounted in clear acrylic, after which they were sectioned using a cutting device (Pressi-T201A, France) to remove the enamel and expose the outer third of the coronal dentin. To create a smear layer, a grinding machine (Dorsa, Iran) was utilized, along with sandpapers of varying grit sizes (100, 400, and 1000). The dentin surfaces of the samples were ground using sandpaper of increasing fineness, with each grit applied for 60 seconds.

The samples were then divided into five groups of negative control, positive control, Diode laser, Remin pro, and combination treatment (N=9 in each). All samples except those in the negative control group were immersed in a 6% citric acid solution (Kimiya Pars, Iran) for 180 seconds to expose the dentinal tubules. A rectangular area measuring 3 × 8 mm^2 was marked on the cross-section of the cervical third of each sample to identify the location for treatment processes and imaging. Samples in each group underwent the specified surface treatment:

Group 1: Negative control. Teeth in this group had a smear layer formed on their surface and did not undergo any surface preparation.

Group 2: Positive control. Teeth in this group also had a smear layer, but their dentinal tubules were exposed using 6% citric acid, without any additional treatment.

Group 3: Diode laser. The teeth in this category underwent irradiation using a diode laser operating at a wavelength of 980 nm (Simpler, Doctor Smile; Italy). The laser utilized a fiber size of 300 micrometers (as detailed in the Supplementary section), and the handpiece had a diameter of 1 cm. An energy density of 25.48 J/cm^2 was applied in a sweeping motion across nine teeth, utilizing a power of 1 W/CW (continuous wave) for a duration of 20 seconds. This procedure was conducted without contact, maintaining a perpendicular angle to the tooth surface from a distance of 1 mm to avoid contamination of the dentin and prevent burning of the dentin surface. The laser tip was swept across the area until each location received irradiation only once.

Group 4: Remin Pro. This product was applied twice daily for three minutes each time, with a 12-hour interval, over the course of one week, as instructed by the manufacturer.

Group 5: Combination treatment. In this group, Remin Pro was applied once to the tooth, followed by diode laser irradiation as described in Group 3.

The samples were then prepared for the imaging using scanning electron microscopy (SEM). First, they were dried using a heater set to a temperature of 70 degrees Celsius. Next, the samples were coated with a 10-nanometer-thick layer of gold before being imaged at a magnification of 2000x. The images obtained from the SEM were then analyzed using Adobe Photoshop 2022 (version 23.5.1.724). In the analysis, five circles with a diameter of 20 micrometers (at 2000x magnification) were randomly selected from the four corners of each image, as well as from the center. The cross-sectional area of the open dentinal tubules was evaluated in pixel units, and the average percentage of open dentinal tubules across the images was calculated. For data analysis, R software (version 1.1.4) was used. The openness of the dentinal tubules was assessed using one-way analysis of variance (ANOVA) to investigate the relationship between the tubule openness and three different treatment methods. Post-hoc analysis was conducted using the least significant difference (LSD) test. A statistical significance level of less than 0.05 was considered significant.

Results

The mean and standard deviation of the percentage of open dentinal tubules in each group are shown in Table 1. The analysis of the data regarding the openness of dentinal tubules in the study groups revealed statistically significant differences, as determined by a one-way ANOVA ($P<0.001$). The positive control group exhibited the highest average percentage of open dentinal tubules, whereas the diode laser group combined with Remin-Pro showed the lowest percentage (Figure 1). SEM images of samples in each group are presented in Figure 2.

Pairwise comparisons of groups with a post hoc LSD test are presented in Table 2. When comparing the treatment methods to the positive control group, all three treatments demonstrated statistically significant results (P=0.000, 0.017, and 0.000). However, no significant statistical differences were found when the three treatment groups were compared to one another (P = 0.279, 0.935, and 0.733).

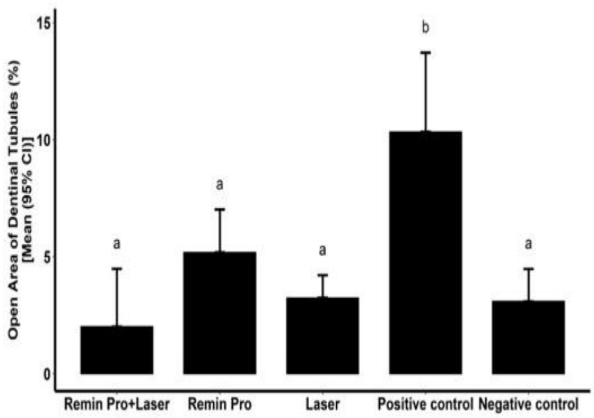

Figure 1: Percentage of openness of dentinal tubules in treatment and control groups.

Table 1- Mean (standard deviation) of the percentage of open dentinal tubules

Groups	Remin.Pro+Laser (N=9)	Remin.Pro (N=9)	Laser (N=9)	Positive control (N=9)	Negative control (N=9)	P-value (one-way ANOVA)
Mean (SD)	2.02 (± 3.78) a*	5.18 (± 2.81) a*	3.25 (± 1.48) a*	10.33 (± 5.18) b*	3.09 (± 2.12) a*	<0.001

*Groups indicated by “a”mean that the statistical results do not differ significantly between these groups; however, the results of the positive control group are significantly different from the other groups and for this reason are shown as”b”.

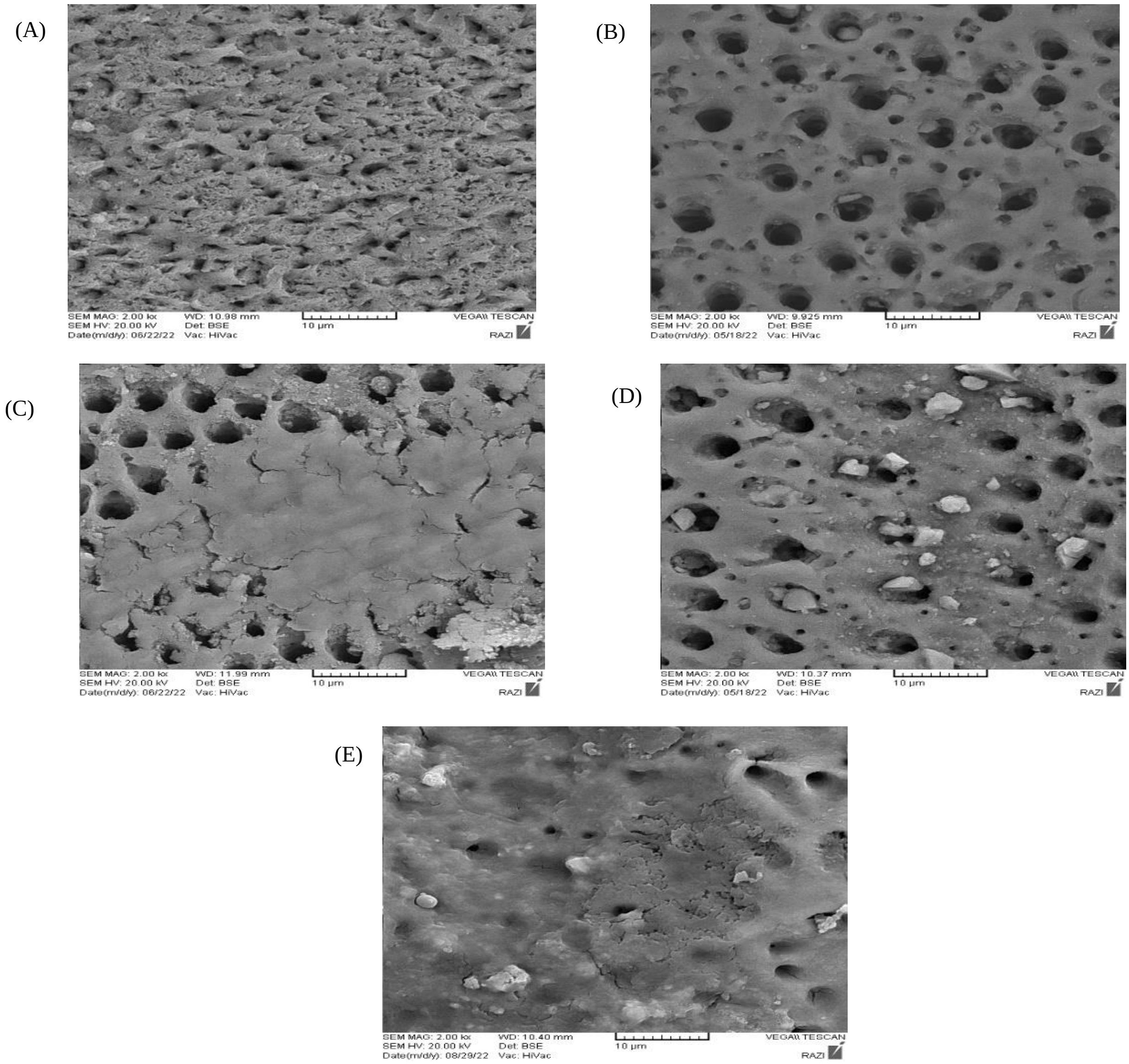

Figure 2: SEM images of dentinal tubules with 2000 magnification in A: Negative control group (without smear layer removal); B: Positive control group (after smear layer removal); C: Diode laser group; D: Remin-Pro group; and E: Combination of diode laser and Remin-Pro

Table 2 – P values in pairwise comparisons of groups with LSD test (P<0.05)

Groups	Remin. Pro	Laser	Positive control	Negative control
Remin.Pro +Laser	0.279	0.935	0.000*	0.959
Remin.Pro		0.733	0.017*	0.676
Laser			0.000*	1.0000
Positive control				0.279

Discussion

Dental sensitivity is one of the most common dental problems caused by exposed dentin reacting to thermal, tactile, and chemical stimuli [3]. With an aging population and a greater retention of natural teeth compared to the past, there has been a noticeable increase in exposed root surfaces and dental abrasions [14]. The surface area of dentin is significantly greater at the dentin-enamel junction (DEJ) and the cementodentinal junction compared to the side of the pulp cavity. As odontoblasts create dentin while moving inward towards the pulp, the tubules are compressed closer together. The quantity of tubules increases from the DEJ towards the pulp. Additionally, the lumen of the tubules varies from the DEJ to the pulp surface. In coronal dentin, the average diameter of dentinal tubules at the DEJ is smaller, but this measurement increases as they approach the pulp [15]. In this investigation, it was aimed to investigate the therapeutic effects of Diode laser treatment and Remin-pro on occlusion of dentinal tubules; therefore, the cervical region was selected. The analysis was conducted with a focus on this specific region, since this area is particularly prone to sensitivity due to various factors, including gingival recession and exposure of dentin. Despite numerous studies evaluating various methods to reduce dentin sensitivity, most treatments have been proven to be ineffective or with limited impact.

Research by Rimondini et al. indicated that premolars and incisors are more sensitive to air and probe stimulation compared to molars [16]. Furthermore, Wichgers and Emery reported that 18% of the adult population experiences dentin hypersensitivity, with the highest prevalence in premolars, followed by canines, incisors, and molars, particularly in the buccal regions of the teeth [17]. In the present study, human premolar teeth were used as samples.

In a study conducted by Saluja et al., the effects of Nd:YAG, CO2, and diode lasers were compared, along with an assessment of morphological changes on the dentin surface. The results indicated that the Nd:YAG laser had the most significant impact, followed by the CO2 laser, while the 810 nm diode laser exhibited the least effect. Notably, the study found that morphological changes, such as cracks, fissures, and burn marks on dentin were more pronounced with the use of CO2 laser compared to the other lasers [18]. Given the adverse effects of the CO2 laser on dentin, its use for treating dentin hypersensitivity does not seem justified[19]. Considering the infrequent use of Er:YAG and Nd:YAG laser devices in dental offices, diode laser was utilized in this study. The primary advantage of the diode laser is its widespread use in dental practices. Its compact size, affordability, portability, and effectiveness on both hard and soft tissues have made it the mostly used type of laser in dental offices. In contrast, the high cost of other laser devices has limited their application[20]. The results of the present study demonstrated that occlusion of dentinal tubules using a 980 nm, 1 W diode laser with continuous irradiation was statistically significant when compared to the control group, confirming that this laser is potentially effective in reducing dentin hypersensitivity.

In another laboratory study, Solati et al. investigated the occlusion of dentinal tubules with nanobioglass in combination with diode and Nd:YAG lasers to reduce permeability and dentin sensitivity using SEM. They found that irradiation with either Nd:YAG or diode lasers, whether used alone or in conjunction with nanobioglass, had a significant impact on occluding the dentinal tubules[21]. Additionally, Modabber et al. examined the effectiveness of a new protocol for reducing dentin sensitivity using a diode laser and graphite paste. They treated 184 patients by first applying graphite paste to the dentin and then using a continuous-mode diode laser at an output power of 1 W in a non-contact manner. Their findings indicated that immediately following treatment the pain was significantly reduced, and average pain levels remained stable over a 6-month follow-up period [10].

The use of lasers in treating dentin hypersensitivity has shown conflicting results, with some attributing the observed therapeutic effects to a placebo effect. In a study conducted by Gholami et al., it was found that diode lasers had minimal impact on altering the diameter of dentinal tubules. They mentioned that due to poor absorption in hard tissues, these lasers were unable to achieve effective tubule occlusion. The authors concluded that CO2, Er,Cr:YSGG, and Nd:YAG lasers could effectively melt peritubular dentin, thereby occluding the openings of dentinal tubules and alleviating symptoms of sensitivity in patients. They noted that the mechanism of action for the diode laser involved the suppression of neural transmission; however, it is essential to highlight that a 2W (high-power) diode laser was used in their investigation [22].

Regarding the Remin Pro material, Asmaa et al. compared it with other products in a laboratory study and concluded that the nano-hydroxyapatite (Remin Pro) group exhibited nearly complete sealing of the dentinal tubules, with the lowest average surface area of opened segments. The differences among the studied groups were statistically significant, with sodium fluoride treatment resulting in the formation of semi-obliterated dentinal tubules. The open areas of dentinal tubules in the sodium fluoride group were statistically comparable to those in the self-assembling peptide group [23]. Ghafournia et al. compared the effectiveness of 5% sodium fluoride varnish, Remin Pro (containing nano-hydroxyapatite and fluoride), MI Paste (containing CPP-ACP), and GC Tooth Mousse (also containing CPP-ACP). They found that both CPP-ACP paste and Remin Pro paste effectively reduced dentin permeability [13]. In the present study, Remin Pro was shown to occlude dentinal tubules effectively with a statistically significant difference compared to the control group. Considering the limitations of the present in vitro study, the Remin Pro desensitizing agent successfully altered the micromorphology of the dentin surface, leading to partial or complete occlusion of the dentinal tubules, with only 5.18% (± 2.81) of the tubules remaining open. This suggests that Remin Pro is a promising biomimetic material for managing dentin hypersensitivity.

To the authors' knowledge, previous studies have not examined the concurrent effects of various lasers in combination with Remin-Pro. This study results indicated that the combined use of Remin-Pro and a diode laser resulted in

an increased dentinal tubule occlusion compared to using either method alone; however, this increase was not statistically significant.
In future studies, it is recommended that researchers evaluate the long-term efficacy of diode laser application, Remin-Pro, and their combination in occluding dentinal tubules, preferrably in an in vivo setting. Additionally, investigations should be conducted to assess the side effects of diode laser application, Remin-Pro, and their combination on reducing the diameter of dentinal tubules.

Conclusion

According to the results of the present study, both the diode laser and Remin-Pro, used individually or in combination, effectively occluded dentinal tubules. However, none showed any superiority over the others.

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Ethical Approval Code: The current study was approved by the Ethics committee of Qazvin University of Medical Sciences (IR.QUMS..REC.1400.428).

Informed Consent Statement. Samples in this study were the priorly extracted teeth; therefore, it didn't require an informed consent

Data Availability Statement: All data is available from the corresponding author upon request.

Using AI: In the study, AI was not used in any stages.

Conflict of Interest: The authors report no conflict of interest.

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