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Evaluation of the Effects of 810 nm Diode Laser Alone and in Combination With Gluma© and Chromophore on Dentinal Tubule Occlusion: A Scanning Electron Microscopic Analysis

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Abstract

Introduction: Tooth hypersensitivity is among the most common patient complaints caused by the response of exposed dentin to external stimuli. No definite treatment has been suggested so far for dentin hypersensitivity (DH). This study aimed to compare the efficacy of the diode laser alone and in combination with Gluma and chromophore in occluding opened dentine tubules and the treatment of DH.

Methods: This in vitro study was conducted on 30 extracted human third molars kept in 0.1% thymol solution. The teeth were mounted in transparent acrylic resin and the buccal enamel was cut to expose the outer third of dentin. The samples were then divided into 6 groups of negative control (no smear layer removal), positive control (smear layer removal with 6% citric acid application), 810 nm diode laser irradiation (energy density 55.55 J/cm^2, 1 W for 20 seconds in a continuous mode), chromophore (1 mg/mL indocyanine green, ICG) plus diode laser irradiation (1 W, 20 seconds), Gluma plus diode laser irradiation, and Gluma + chromophore + diode laser. Dentinal tubules were evaluated under a scanning electron microscope at x2000 magnification. The mean percentage of the obstruction of dentinal tubules was reported as mean and standard deviation. Considering the normal distribution of the data, two-way ANOVA was applied to compare the efficacy of treatments, and an independent-samples *t* test was used for pairwise comparisons at P<0.05 level of significance.

Results: The highest mean percentage of the obstruction of dentinal tubules was noted in the diode laser/chromophore/Gluma group (65.68±12.31%) while the lowest value was noted in the diode laser/Gluma group (24.33±5.90%). Pairwise comparisons revealed significant differences between all groups ($P<0.05$) except for the difference between the laser/Gluma/chromophore and laser/chromophore groups ($P=0.20$).

Conclusion: It seems that chromophore increases the efficacy of the diode laser for the obstruction of dentinal tubules.

Keywords: Dentin hypersensitivity; Laser; Dentin desensitizing agents; Indocyanine green.

Introduction

Many factors such as abrasion, attrition, erosion and periodontal disease can cause dentin hypersensitivity (DH), which is different from dental defects or pathologies. Exposure of dentin occurs as a result of the removal of the enamel for any reason or denuded root dentin due to the loss of cementum and periodontal tissue.[1,2]

Considering the increased proportion of older people retaining their natural teeth, more root surfaces are exposed due to gingival recession and periodontal surgery. As a result, the incidence of DH is on the rise.[3]

Several theories have been suggested to explain the mechanism of DH, such as (I) direct innervation of dentin, (II) odontoblast receptor theory, and (III) hydrodynamic theory which is the most popular.[4] According to the hydrodynamic theory, stimulants applied to dentin cause the movement of the intratubular fluid. This movement causes a mechanical change in the nerve endings at the pulp-dentin interface where the pain is felt. Thus, any technique that decreases the movement of intratubular fluid or dentin permeability can decrease DH. Microscopically, the number and size of open dentinal tubules determine the degree of DH.[5,6] According to the hydrodynamic theory, the higher the number of open dentinal tubules and the greater the diameter of tubules, the faster the transfer of signals and the consequent hypersensitivity would be.[7,8]

Desensitization of teeth is performed in 2 ways:

1. Natural desensitization: Exposed dentin is not always sensitive. Desensitization of teeth may occur naturally.

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Thus, hypersensitivity greatly decreases over time. Dental calculus is periodontally unfavorable but can help in the obstruction of dentinal tubules. Salivary proteins cover the external dentin surface while plasma proteins cover the internal dentin surface and obstruct the dentinal tubules.[9]

2. Treatments performed by dentists: These treatments include the application of corticosteroids, oxalates, potassium and fluoride salts, iontophoresis, varnishes, dentin bonding agents, and lasers.[10]

The laser device was first discovered by Maiman in 1960 and has created new promises in dentistry. One of its applications is in the treatment of DH.

The laser decreases DH via 2 mechanisms: (1) The laser directly affects the electric conduction of nerve fibers present in dental pulp. (2) The obstruction of dentinal tubules by melting.[11,12]

The diode laser is a low-intensity laser with 3 wavelengths of 780, 810, 900 nm used in the treatment of hypersensitivity. This laser was first used at a 780 wavelength by Matsumoto et al in 1985 to treat hypersensitivity, and its therapeutic effect is estimated to be 85%-100%.[11]

Gluma© desensitizer, which is composed of glutaraldehyde and hydroxyl ethyl methacrylate (HEMA), is among the commercially available desensitizing agents. Glutaraldehyde causes the coagulation of proteins and amino acids in the tubules and is also an effective disinfecting agent. HEMA can also effectively obstruct the dentinal tubules. Gluma© does not compromise the bond to dentin. However, it contains glutaraldehyde, which may damage the gingiva following long-term contact. Also, due to the presence of HEMA in its composition, Gluma© can cause contact dermatitis.[13]

Chromophore is an organic unsaturated molecule that contains a group of single or multiple chain bonds. It changes the light absorbance to lower frequencies and longer wavelengths. Indocyanine green (IG) has an absorption peak near 800 nm, which is near to the emission maximum of commercially dental diode lasers. Thus, it has greater penetration depth into periodontal pockets.[14] The application of the laser and glutaraldehyde has been evaluated separately for the treatment of DH in many previous studies.[15-17] On the other hand, chromophore (IG) is commonly used in photodynamic therapy.[18] However, its efficacy for the treatment of DH has not been evaluated. This study aimed to compare the efficacy of the 810 nm diode laser alone and in combination with glutaraldehyde (Gluma©) and chromophore (IG) in occluding opened dentine tubules and the treatment of DH.

Materials and Methods

Preparation of Samples

This in vitro study was performed on 30 human third molar teeth extracted for impacted reasons. The teeth were sound and collected for three months.[18] The teeth were stored in 0.1% thymol at 4°C to prevent microbial growth.[19]

The teeth were first mounted in transparent acrylic resin and sectioned (CNC, Nemophanavaran Pars, Iran) to eliminate the buccal enamel and expose the outer third of dentin (coronal region). Next, 100, 400 and 1000-grit abrasive papers were used with a polishing machine (Dorsa, Iran) to create the smear layer. Dentin surfaces (6 × 6 mm) were ground using coarse to fine grit abrasive papers for 60 seconds. For the exposure of dentinal tubules, all samples except for the negative control group (5 teeth that did not undergo surface preparation) were immersed in 6% citric acid (Kimia Pars, Iran) for 90 seconds.[20]

Therapeutic Interventions

The samples were randomly divided into 6 groups of 5 (n=5) as follows:

- Group 1 (negative control): In this group, after the creation of the smear layer, the samples did not undergo any surface treatment.
- Group 2 (positive control): Dentinal tubules in this group were exposed using 6% citric acid. No other intervention was performed.
- Group 3 (diode laser): After surface treatment, the teeth were subjected to 810 nm diode laser irradiation (Wuhan Gigaa Optronics Technology Co,LTD, China) with 1W power in a continuous mode for 20 seconds. The fiber diameter was 320 μm and the irradiation was done in sweeping motion with 1 mm distance from the surface.
- Group 4 (diode laser/chromophores): After preparation with citric acid, the samples were coated with one layer of chromophore with approximately 20 μm thickness (ICG) and 1 mg/mL concentration and were then subjected to diode laser irradiation with output power of 1 W for 20 seconds. The diameter of the tip was 320 μm and irradiation was done 1 mm above the surface.
- Group 5 (diode laser/Gluma©): After preparation, Gluma (Heraeus Kulzer, Dormagen, Germany) was applied to the surface of the samples and they were then irradiated with the diode laser as explained for group 3.
- Group 6 (diode laser/Gluma©/chromophores): After preparation, Gluma was applied to the teeth followed by chromophore and then the samples were subjected to diode laser irradiation as in group 3.

The total energy density for the total area of the tooth was 55.55 J/cm^2.

Table 1 shows the characteristics of the laser and materials used in this study.

Scanning Electron Microscope

The samples were prepared for Scanning electron

Table 1. Materials and Devices Used in This Study

Diode laser	1 W , energy density for total area of tooth was 55.55 J/cm^2	20 seconds, continuous mode, sweeping motion	Wuhan Gigaa Optronics Technology Co, LTD, China
Gluma	5% glutaraldehyde and 35% HEMA	The tooth surface was dried, Gluma was applied with a brush, after 30-60 seconds, completely dried and then rinsed	Heraeus Kulzer, Dormagen, Germany
Indocyanine Green	1 mg/2 mL	Applied to the surface with an approximate thickness of 20 µm using a microbrush	EmunDo, A.R.C Laser

microscope (SEM analysis). They were sputter-coated with one layer of gold with 10 nm thickness. The samples were then inspected under an SEM (VEGA TS5136MM, TESCAN, Czech Republic) at x2000 magnification.

Statistical Analysis

To assess the mean percentage of the obstruction of dentinal tubules, SEM micrographs taken at ×2000 magnification were analyzed in Adobe Photoshop CC 2015 v. 16.0 software. On each SEM micrograph, five circles with 3-centimeter diameter were randomly chosen at the four corners and center of the image, and the cross-sectional area of obstructed and partially obstructed dentinal tubules was calculated in pixels. Data were analyzed using SPSS version 22.

The mean percentage of the obstruction of dentinal tubules was reported as mean and standard deviation. Assuming the normal distribution of the data, two-way ANOVA was applied to compare the groups in terms of the efficacy of the treatments. Since the interaction effect of Gluma© and chromophore was significant, pairwise comparisons were carried out using an independent-samples *t* test. $P<0.05$ was considered statistically significant.

Results

Two-way ANOVA showed that the interaction effect of Gluma© and chromophore was significant. The highest mean percentage of the obstruction of dentinal tubules was noted in the diode laser/chromophore/Gluma group (65.68 ± 12.31%) while the lowest value was noted in the diode laser/Gluma group (24.33 ± 5.90%) (Figures 1 & 2; Table 2).

Also, the effect of Gluma and chromophore on the percentage of the obstruction of dentinal tubules was separately analyzed using an independent-samples t-test. The pairwise comparisons of the groups showed a significant difference between the laser and laser/chromophore groups ($P=0.004$), and the laser/chromophore group was superior to the laser group in this regard. The comparison of the laser and laser/Gluma groups revealed that the laser group was superior to the laser/Gluma group ($P=0.002$). Significant differences were noted between the laser/Gluma/chromophore and laser/Gluma groups ($P<0.001$) but no significant difference was noted between the laser/Gluma/chromophore and laser/chromophore groups ($P=0.20$, Table 3).

Discussion

Tooth hypersensitivity is among the most common and most painful dental complaints and depends on many factors. The use of lasers for the treatment of DH has been associated with controversial results and some authors believe that its therapeutic effects are related to

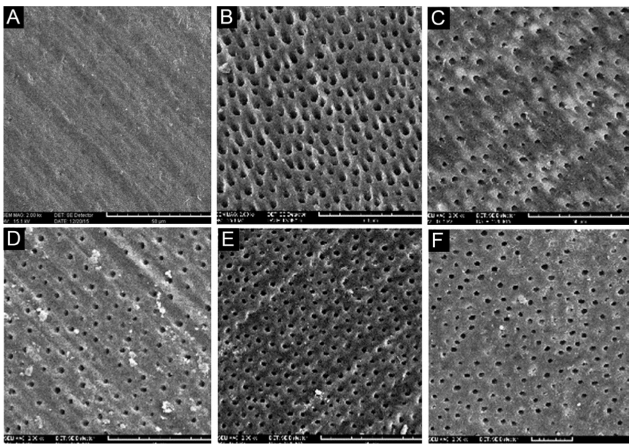

Figure 1. (A) SEM micrograph shows the dentinal tubules without removing the smear layer and no surface treatment at x2000 magnification. (B). SEM micrograph shows the exposed dentinal tubules using 6% citric acid and no other surface treatment at x2000 magnification. (C) SEM micrograph shows the dentinal tubules subjected to 810 nm diode laser irradiation (x2000 magnification). (D) SEM micrograph shows the dentinal tubules subjected to 810 nm diode laser irradiation after coating with one layer of chromophore. (E) SEM micrograph shows the dentinal tubules after the application of Gluma© and the 810 nm diode laser. (F) SEM micrograph shows the dentinal tubules after the application of Gluma©, chromophore and the 810 nm diode laser.

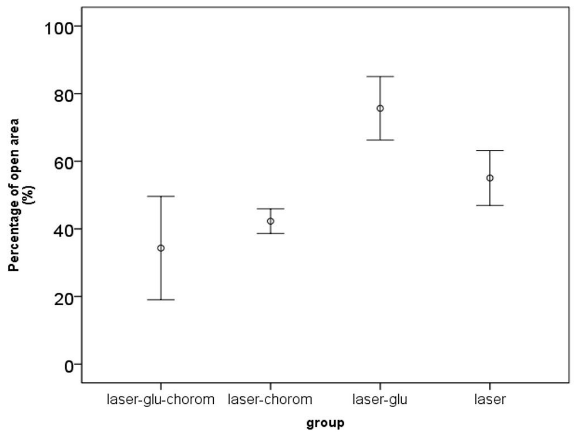

Figure 2. Comparison of the Percentage of the Obstruction of Dentinal Tubules in the Groups.

the placebo effect.[21] However, some studies have reported that laser irradiation alone or in combination with other chemical agents such as Gluma©, sodium fluoride, and potassium nitrate yield stable results equal or superior to those of chemical methods for the treatment of DH.[22-24]

This study aimed to assess the efficacy of the diode laser alone and in combination with other chemical agents in occluding opened dentine tubules and the treatment of DH. To simulate the clinical setting, we tried our best to create a smear layer with uniform thickness on all samples. Since chromophore (IG) is believed to play an efficient role in the absorption of laser energy, particularly the diode laser, we used chromophore (IG) for the first time to increase the efficacy of laser in the obstruction of dentinal tubules. The results showed that the laser/chromophore combination was more effective than other groups in the obstruction of dentinal tubules because no significant difference was noted between the laser/Gluma/chromophore and laser/chromophore groups ($P=0.20$). It seems that chromophore increases the absorption of diode laser energy. IG, in contrast to other chromophores which play a photochemical role, has a photothermal effect. Thus, it seems that it enhanced the mild melting of the dentinal tubules. Considering the fact that the IG photosensitizer is activated with the 810 nm diode laser, this combination has been successfully used in photodynamic therapy for endodontic and periodontal purposes.[25,26] No previous study has evaluated the efficacy of the combination of the diode laser and IG in occluding opened dentine tubules and the treatment of DH. In the current study, chromophore (IG) was used to increase the efficacy of the diode laser. The percentage of the obstruction of the dentinal tubules was 58%, which indicated the optimal efficacy of chromophore when used in combination with the diode laser for increasing the absorption of laser energy and the obstruction of dentinal tubules. Umana et al[27] stated that the 810 nm diode laser caused mild, irregular and random melting of peritubular and intertubular dentin and led to the partial obstruction of dentinal tubules. However, they used graphite paste as chromophore, which was different from the chromophore used in our study (IG). In their study, half of the samples were coated with chromophore (graphite paste) and subjected to 810 and 980 nm diode laser irradiation with different powers. The results showed that graphite paste increased heat generation and accelerated the melting of dentinal tubules. Consequently, the diameter of the open tubules decreased or they were completely occluded. However, the application of chromophore (graphite paste) was effective when combined with 0.8 and 1 W power of the diode laser, and the samples were destructed when 1.6 and 2 W power of the laser was used.[27]

In the current study, the diode laser with 1 W power was used in combination with chromophore (IG) and yielded results similar to those of Umana et al.[27] No crack or destruction was noted under the SEM. Evidence shows that the diode laser can obstruct the dentinal tubules with different mechanisms. Controversy exists regarding the mechanism of the effect of the laser on dentinal tubules and the treatment of DH. However, the main advantages of the diode laser include small size, affordability, and portability. Thus, we used the diode laser in this study. Gholami et al[28] stated that the diode laser had insignificant efficacy in changing the diameter of dentinal tubules

Table 2. The Mean and Standard Deviation of the Percentage of the Obstruction of Dentinal Tubules in the Groups

Group	Mean	SD	SE
L (n=5)	44.94	6.54	2.92
L- CR (n=5)	57.72	2.94	1.31
L-G (n=5)	24.33	5.90	2.94
L-G-CR (n=5)	65.68	12.31	5.50

L: Laser, G: Gluma, CR: chromophore, SD: standard deviation, SE: standard error.

Table 3. Pairwise Comparisons of the Groups

	Mean Difference	Std. Error Difference	95% CI		*P* Value
			Lower	Upper	
L vs. L-CR	12.80	3.20	5.40	20.20	0.004
L vs. L-G	-20.60	4.20	-30.60	-10.70	0.002
L-G-CR vs. L-CR	8.00	5.70	-5.10	21.00	0.20
L-G-CR vs. L-G	41.30	6.80	25.40	57.30	<0.001

L: Laser, G: Gluma, CR: chromophore.

and due to its poor absorption into the hard tissues, it could not efficiently obstruct the tubules. They showed that different lasers had different efficacy in sealing the tubules. The assessment of the mean diameter of dentinal tubules showed that Nd:YAG, CO2 and Er:Cr:YSGG lasers melted the peritubular dentin and completely or partially occluded the dentinal tubules. Thus, DH decreased. However, they explained the mechanism of the action of the diode laser to be through the inhibition of signal transfer. But they used a 2 W diode laser (high-level laser) and did not use chromophore.[28] Their results regarding no effect of the diode laser on the diameter of dentinal tubules were different from ours, which is due to the aforementioned explanations. Osmari et al[29] compared the efficacy of desensitizing agents (fluoride varnish, potassium oxalate, two-step self-etch adhesive systems and diode laser) for the obstruction of dentinal tubules and reported that desensitizing agents can cause partial or complete obstruction of dentinal tubules via different mechanisms. The laser caused the partial obstruction of dentinal tubules via melting and contraction of dentin.

In the current study, the percentage of the obstruction of dentinal tubules in the diode laser group was found to be 45%. Thus, it caused the partial and complete obstruction of a number of dentinal tubules. In this study, the efficacy of the combination of the diode laser and Gluma was evaluated, which had no superiority over the use of the laser alone and Gluma decreased the efficacy of the laser. No in vitro study was found on the combination of the 810 nm diode laser and Gluma; thus, we compared our findings with those of clinical studies.

Kara et al[30] in a clinical study, separately compared the efficacy of the 940 nm diode laser and Gluma for the treatment of DH following tooth preparation. They indicated that none of the tested modalities had any superiority over the other. In another clinical study, Raut et al[31] compared and evaluated the effect of low-power diode lasers with and without topical application of stannous fluoride (SnF2) gel and showed that diode lasers alone and in combination with 0.4% SnF2 were effective in the treatment of DH. The results of the aforementioned clinical studies were in agreement with our findings. Our study showed that the diode laser was superior to the combination of diode laser/Gluma for the obstruction of dentinal tubules. Also, the combination of diode laser/chromophore was not significantly different from laser/Gluma/chromophore in terms of efficacy. Thus, it seems that the negative effect of Gluma in the latter combination was compensated by chromophore.

Conclusion

Considering the limitations of this study, it may be concluded that:

1. The diode laser group was only superior to diode laser/Gluma in terms of the obstruction of dentinal tubules and it is possible that Gluma© blocks the laser energy.
2. The pairwise comparison of the laser/Gluma/chromophore and laser/chromophore groups revealed no significant difference. It seems that the negative effect of Gluma© was compensated by chromophore.
3. It seems that the combination of the laser and chromophore is effective in the treatment of DH.

Ethical Considerations

The study was approved in the ethics committee of Tehran University of Medical Sciences (code: IR.TUMS.REC.1394.850).

Conflict of Interests

The authors declare that they have no conflict of interest.

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