ORIGINAL RESEARCH

Combined Effects of Glutaraldehyde-based Desensitizer and Nd: YAG Laser on Dentinal Tubules Occlusion

Alya H Alzarooni[1], Hatem M El-Damanhoury[2], Soumya S Aravind[3], Betul Rahman[4]

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Abstract

Aim: The aim of this study was to assess the impact of Nd:YAG laser, glutaraldehyde-based desensitizer (GD), or their combination on occluding dentinal tubules.

Materials and methods: Fifty dentin samples were obtained from non-carious human third molars and randomly divided into five groups ($n = 10$): (1) Control group treated with 37% phosphoric acid, (2) GD group, (3) Nd:YAG laser group (1064 nm, 100 µs, 10 Hz, 300 µm fiber, 1 W power, 100 mJ energy, and 85 J/cm^2 energy density), (4) GD followed by Nd:YAG laser group, and (5) Nd:YAG laser followed by GD group. Scanning electron microscopy (SEM) was used to capture five images from each sample for analysis of dentinal tubules using Image J software. SEM/EDX elemental analysis was performed to determine the main mineral contents. Data analyzed using one-way ANOVA and Tukey's *post hoc* test for statistical comparisons.

Results: Laser and combination groups showed a significant decrease in dentinal tubule counts compared with the control and GD groups ($p < 0.0001$). There were no significant differences in open dentinal tubule counts between the control and GD groups, as well as between the laser and combination groups. However, significant differences were observed in the total area, average size of the tubules, and percentage area between the control group and the treatment groups (GD, laser, GD + laser, laser + GD). No significant difference was found in the Ca/P ratio between the tested groups.

Conclusion: The use of Nd:YAG laser alone or in combination with GD was more effective in occluding dentinal tubules compared to GD alone.

Clinical significance: This study has shown that Nd:YAG laser alone and in combination with GD has superior dentinal tubule occlusion *in vitro*. Its clinical use in the treatment of dentinal hypersensitivity may overcome the drawback of conventional treatment approaches for dentin hypersensitivity needing repeated applications to achieve continuous relief from pain since acidic diet and toothbrushing result in the continuing elimination of precipitates and surface coatings.

Keywords: Dentinal hypersensitivity, Dentinal tubules, Desensitizing agents, Nd:YAG laser scanning electron microscopy.

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Introduction

Dentinal hypersensitivity (DH) is a commonly occurring dental problem.[1] It is characterized by unique short, sharp pain evolving from the exposed dentin in response to external stimuli, which usually is thermal, tactile, evaporative, osmotic, or chemical. The most agreed theory concerning the mechanism of DH is the hydrodynamic theory by Brännström,[2] suggesting that pain-eliciting stimuli would cause fluid movement across the dentinal tubules, and accordingly, stimulate the nerves around odontoblasts resulting in pain and discomfort.[3] Two main DH treatment strategies have been suggested for dentin desensitization. One is the occlusion of dentinal tubules to reduce dentinal fluid movement; the other is reducing the dental sensory nerves activity.[4,5]

Treatments of DH have included a variety of physical and chemical agents that can be used at home or by a clinician. For example, calcium compounds, cavity varnishes, oxalates, fluoride-based interventions, strontium chloride, and lasers. GLUMA desensitizer (GD) is a resin material composed of 5% glutaraldehyde, 35% 2-hydroxyethyl-methacrylate (HEMA), and purified water. Based on the manufacturer, GD acts via precipitating plasma proteins that decrease the permeability of dentin and internally block the dentinal tubules preventing the fluid flow inside them.[6] Glutaraldehyde in GD is a biological adhesive. Its role in successful dentinal tubule blockage was reported as by reacting with proteins in tubular fluid and causing precipitation. In addition, HEMA was shown to be an effective hydrophilic monomer for dentin bonding. However, studies on the role of GD on the dentinal tubule occlusion are controversial. Some studies reported partial occlusion of dentinal tubules and bacterial contamination by GD.[7,8]

In the mid-1980s, the usage of laser beams in DH treatment was proposed for the first time.[9] The lasers used for dental hypersensitivity

[1]Department of Dentistry, Emirates Health Services, Sharjah, United Arab Emirates

[2,4]Department of Restorative Dentistry, College of Dental Medicine, University of Sharjah, Sharjah, United Arab Emirates

[3]Dental Biomaterials Research Group, Research Institute for Medical and Health Sciences, University of Sharjah, Sharjah, United Arab Emirates

Corresponding Author: Betul Rahman, Department of Restorative Dentistry, College of Dental Medicine, University of Sharjah, Sharjah, United Arab Emirates, Phone: +97165057365, e-mail: brahman@sharjah.ac.ae

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treatment are usually divided into two groups: low-power lasers (He–Ne and GaAlAs lasers), and high-power lasers (Nd:YAG, Er:YAG, Er,Cr:YSGG, and CO_2 lasers).[10] The odontoblasts' cellular metabolic activity increases as a result of the laser photobiomodulating effect on the dental pulp. It obliterates the dentinal tubules to increase the production of tertiary dentin.[11] A study evaluates the efficacy of three different lasers as dentin desensitizers Nd:YAG (1064 nm, 100 mJ/pulse, 15 Hz, 100 seconds), Er:YAG (2940 nm, 60 mJ/pulse, 2 Hz, 20 seconds), and GaAlAs (Diode; 808 nm, 100 mW, 20 seconds). The Nd:YAG laser irradiation has shown to be more efficient than the Er:YAG laser and the diode laser in treating DH.[12] Nd:YAG laser was shown to block the dentinal tubules by liquefying and re-hardening the dentin without causing any cracks on the dentin surface. Formation of a 4 µm thick obstructive layer created by Nd: YAG laser irradiation was thought to have a significant role in the reduction of dentinal hypersensitivity.[13]

A combination of laser therapy and desensitizing agents' application has been recommended to enhance the treatment outcomes, and it was reported to clinically reduce hypersensitivity associated with hypomineralization.[14,15] There are no sufficient data in the literature to support combining Nd:YAG laser and glutaraldehyde-based desensitizer (GD) to enhance the occlusion of dentinal tubules as a treatment modality for DH. Therefore, this study aimed to investigate and compare the occluding effect of Nd:YAG laser and GD or their combination on human dentinal tubules by scanning electron microscopic analysis. In addition, elemental analysis was done to determine the main mineral contents in dentin following each tested treatment protocol.

Materials and Methods

Preparation of Samples

Freshly extracted human third molars with no caries were collected from the oral surgery clinic, after obtaining informed consent from patients and obtaining the University of Sharjah Research Ethics Committee approval (REC-20-03-08-02-S), teeth were cleaned and soft tissue remnants were removed with an ultrasonic scaler, then they were stored in 0.1% thymol solution for 2 weeks. Fifty samples were prepared by cutting the enamel from the occlusal surface to expose the dentin surface, and the roots were cut at the cemento-enamel junction utilizing a low-speed water-cooled diamond saw (Isomet 1000, Buehler Ltd, Lake Bluff, IL, USA). Teeth were inspected under a stereomicroscope and samples with cracks or pulp exposures were excluded and replaced with new samples. The occlusal dentin surfaces were polished with 600-grit silicon carbide paper using a semi-automatic polisher (EcoMet 30, Buehler Ltd, Lake Bluff, IL, USA). The samples were cleaned in an ultrasonic water bath. The samples were divided randomly into five groups using the simple random sampling technique (n = 10), The five groups were treated as follows:

1. Group I: Dentin etched with 37% phosphoric acid for 2 minutes, rinsed with double-distilled water and received no treatment – control group.
2. Group II: Etched dentin-treated with gluma desensitizer (GD) as per manufacturer's instructions.
3. Group III: Etched dentin treated with Nd:YAG laser.
4. Group IV: Etched dentin treated with GD followed by Nd:YAG laser.
5. Group V: Etched dentin treated with Nd:YAG laser followed by GD.

Table 1: Laser parameters used in this study

Type of laser	Nd:YAG
Model	Fidelis AT, Fotona, Ljubljana, Slovenia
Wavelength	1064 nm
Pulse widths	100 µs
Emission mode	Very short pulse (VSP)
Repetition rate	10 Hz
Delivery system	Fiber-optic; fiber diameters of 300 µm with R211W: Nd:YAG contact tip handpiece
Average power	1 W
Pulse energy	100 mJ
Focus-to-tissue	Yes
Water irrigation	No
Air and aspirating airflow	No

All the dentin specimens from each group were kept in double-distilled water at 37°C for 1 week before testing.

The method of using GD (Gluma Desensitizer, Heraeus Kulzer, Hanau, Germany) was according to the manufacturer's instructions; the material was applied with a microbrush and left to react for 60 s, air-dried with an oil-free air spray, and then rinsed thoroughly with water. The application was repeated three times. The samples were stored in double-distilled water at 37°C until evaluated.

The laser group was irradiated with Nd:YAG laser (Fidelis AT, Fotona, Ljubljana, Slovenia), the laser operating parameters are listed in Table 1. The exposure was operated from a vertical distance of 2 mm with a sweeping motion and without coolant. All the samples were irradiated by the same operator to standardize the laser application. The laser was applied three times for 60 seconds each and with a resting period of 30 seconds between applications.

All samples were dehydrated in a desiccator for 24 hours at room temperature. Surface morphological analysis was done after sputter coating with 100 Å gold–palladium (EMS 7620 Mini Sputter Coater, Hatfield, PA) to improve the surface conductivity and to reduce the electron charging. Images were captured under different magnifications, using a scanning electron microscope (Tescan VEGA XM variable pressure SEM, Kohoutovice, Czech Republic), which operates at a 20 kV acceleration voltage. Five representative images were captured from each sample at 2000× magnification. The images were further analyzed by two calibrated examiners using the Image J software (Image J 1.52v; NIH, Bethesda, MD, USA) to determine the count, total area, average size, and percentage area of open dentinal tubules. Inter-rater agreement reliability was examined using Kappa coefficient.

SEM-coupled energy-dispersive X-ray (EDX) (Oxford Instruments X-Max 50 EDS detector (LN2 free system), UK) technique was conducted for elemental analysis with both map and point modes at the same operating voltage to determine the calcium and phosphorus contents in the dentin samples of each group.

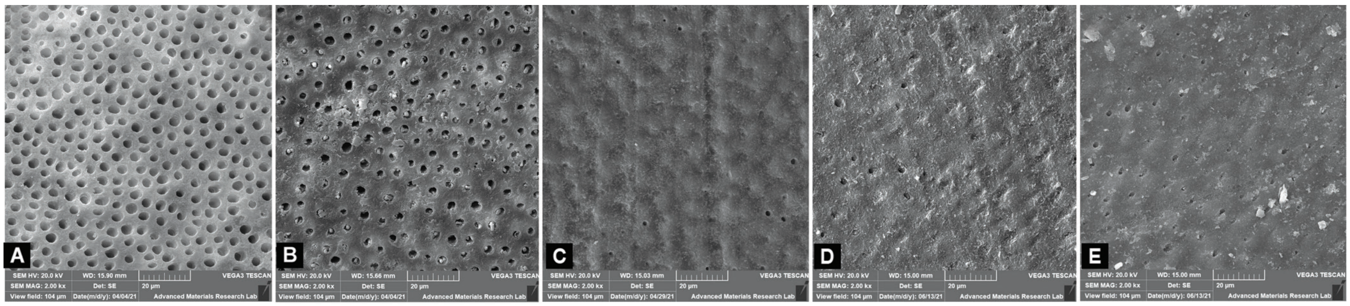
Statistical Analysis

Sample size was calculated using the means comparison formula from the results of the pilot study, to achieve a power of 80% and an alpha error 0.05, using a statistical analysis software (Minitab, State College, Pennsylvania, United States). The effect of the

Table 2: Count, total area, average size and % area of dentinal tubules after treatments application (mean % ± standard deviation)

Treatment	*Count (n)*	*Total area (μm²)*	*Average size (μm)*	*Area (%)*
C	516.48 ± 117.47[a]	3.87 ± 1.70[a]	0.01 ± 0.003[a]	7.06 ± 3.10[a]
GD	465.72 ± 146.64[a]	0.68 ± 0.60[b]	0.001 ± 0.001[b]	1.23 ± 1.09[b]
L	130.4 ± 67.58[b]	0.11 ± 0.08[b]	0.001 ± 0.001[b]	0.20 ± 0.14[b]
GD + L	144.76 ± 49.58[b]	0.11 ± 0.06[b]	0.0007 ± 0.0005[b]	0.20 ± 0.11[b]
L + GD	175.64 ± 63.20[b]	0.12 ± 0.10[b]	0.0008 ± 0.0007[b]	0.23 ± 0.18[b]

Mean values with different superscript letters are statistically significantly different ($p < 0.0001$); C, etched control; GD, gluma desensitizer; L, Nd:YAG laser; GD + L, gluma desensitizer + Nd:YAG laser; L + GD, Nd:YAG laser + gluma desensitizer



Figs 1A to E: Representative SEM photomicrographs (magnification 2000x) of dentin specimens after: (A) 37% phosphoric acid etched (control group); (B) Treatment with GD; (C) Nd:YAG laser irradiation; (D) Treatment with GD then Nd:YAG laser irradiation; (E) Nd:YAG laser irradiation then application of GD

dentin treatment on the results were analyzed using one-way ANOVA followed by Tukey's *post hoc* tests to identify the pairwise differences between experimental groups. A confidence level of 95% was applied for all the statistical tests. All statistical analyses were done using a statistical software (GraphPad Prism 5 version 5.03, GraphPad Software, Inc, Sandiego, CA, USA).

Results

Table 2 presents the count, average size, total area, and percentage area of the open dentinal tubules in each group.

Photomicrographs of the etched group, GD group, laser group, GD + laser group, and laser + GD group are shown in Figure 1. The etched control group (Fig. 1A) showed smooth dentin with opened tubule orifices and no smear layer covering it. Examinations of SEM photomicrographs (Fig. 1B) revealed that the GD group had a smooth homogeneous dentin surface with less exposed dentinal tubules compared with the control group, leaving precipitations on the surface of the dentin and into the dentinal tubules. When laser treatment was done after GD application, the deposits seemed to be fused into the dentin surface. On the other hand, when GD treatment was done after laser, the deposits seemed to precipitate onto the dentin surface. The laser only and combination groups (Figs 1C to E) displayed a homogeneous area with a maximum occluding effect.

The SEM analysis of the specimens displayed that there was significant difference noted in the counts of dentinal tubules for the laser and combination groups in comparison to the control and GD groups ($p < 0.0001$). However, no significant difference ($p > 0.05$) in count was observed between the control (516.48 ± 117.47%) and GD groups (465.72 ± 146.64%) in this investigation (Table 2). Additionally, there were no statistically significant differences ($p > 0.05$) observed in dentinal tubules counts between laser only (130.4 ± 67.58%) and combination groups (144.76 ± 49.58%, 175.64 ± 63.20%).

In the case of the total area, the average size of the tubules, and the percentage area, there seemed to have significant differences between the control and the four treatments (GD, laser, GD + laser, Laser + GD); though no significant difference was found between these four groups.

EDX analysis of major components of the element surface composition of dentin samples' surfaces is shown in Figure 2 and Table 3. There was a statistically significant difference ($p < 0.05$) between the Ca % of control group and the G + L group. However, the calcium-to-phosphorus mineral ratio (Ca/P ratio) of hydroxyapatite in dentin was maintained within similar range between the treatment groups ($p > 0.05$).

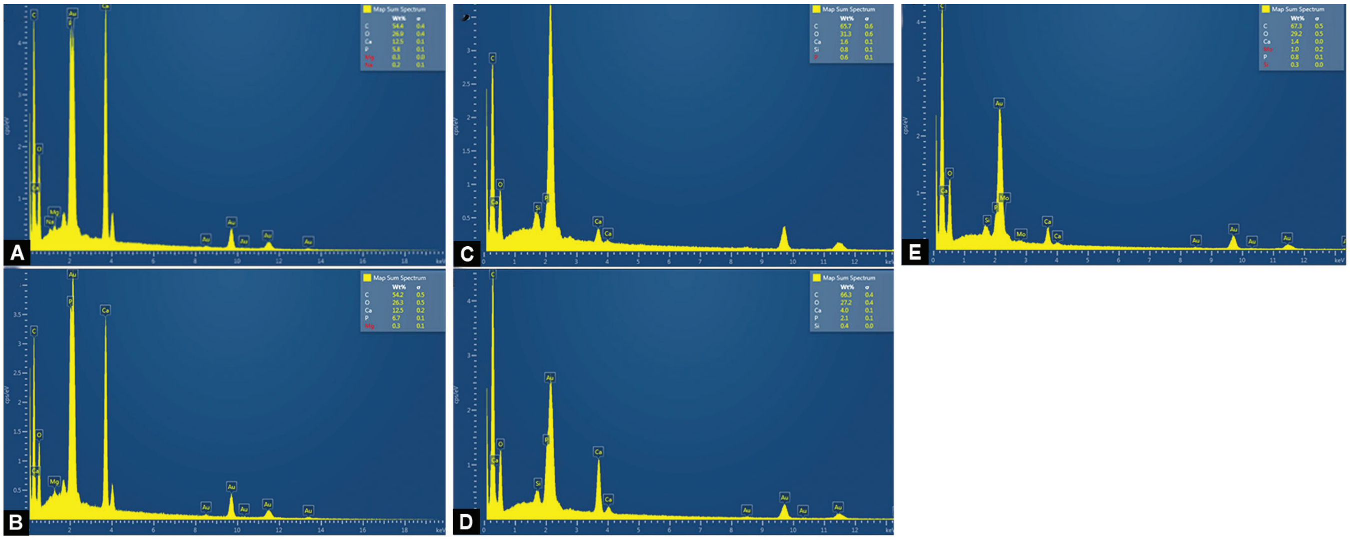
Discussion

The results of the current study showed that Nd:YAG laser was more effectual than GD in occluding the dentinal tubules, and therefore, the null hypothesis was rejected.

It is recognized that the number and diameter of open dentinal tubules subjected to the oral environment are directly linked to dentinal hypersensitivity. The rise in the count and diameter of the open dentinal tubules will lead to an increase in the intensity of stimulation to the pulp.[16,17] Previous SEM studies revealed that the count of open dentinal tubules per unit area in hypersensitive dentin is eight times higher than in non-sensitive dentin and that the tubule diameters are two times wider.[18]

The key principle of success in dentin hypersensitivity treatment is the complete occlusion or at least narrowing of the exposed dentinal tubule. Based on this information, the current study was designed *in vitro* to identify the potential occlusion and desensitizing capability (or potential) of desensitizing agents (GD) and Nd:YAG laser. The effects of GD and Nd:YAG laser irradiation were assessed on certain structural characteristics and contents of dentin, such as changes in the count, average size, total area, and percentage area of dentinal tubules in each group of dentinal tubules and percentage of calcium and phosphorus.

In this study, dentin specimens were etched with 37% phosphoric acid to imitate dentin tubules that were subjected to



Figs 2A to E: Representative energy-dispersive X-ray (EDX) spectra of dentin specimens after: (A) 37% phosphoric acid etched (control group); (B) Treatment with GD; (C) Nd:YAG laser irradiation; (D) Treatment with GD, then Nd:YAG laser irradiation; (E) Nd:YAG laser irradiation then application of GD

Table 3: EDX analysis of major components of element surface composition (wt%) of the dentin surface after treatment with the tested materials

Group	*Ca%*	*P%*	*C%*	*O%*	*Ca/P*
C	12.1[a]	5.48	55.78	26.2	2.21
GD	8.04	4.14	61.44	26.04	1.94
L	7.66	3.94	58.82	29.16	1.94
GD + L	2.84	1.56	66.36	28.8	1.82
L + GD	9.14	5.8	57.04	27.14	1.58

Mean values with superscript letter (a) are statistically significantly different ($p < 0.05$); C, etched control; GD, gluma desensitizer; L, Nd:YAG Laser; GD + L: gluma desensitizer + Nd:YAG Laser, L + GD: Nd:YAG laser + gluma desensitizer

the oral environment. GD utilized in this investigation contained 5% glutaraldehyde and 35% HEMA. Glutaraldehyde reacts with proteins in the dentin fluid resulting in protein precipitation which lead to partial or complete occlusion of the dentinal tubules.[19] As shown in Figure 1B, samples treated with GD revealed some occluded dentinal tubules along with a debris-free dentin surface and certain material precipitations, others showed partial occlusion, and some of the dentinal tubules were open.

Studies conducted using GD have generally stated successful results.[20,21] In a clinical trial, the GD has revealed a statistically significant decrease in sensitivity pain score between baseline and postoperative as well as between the postoperative and the 1-week reactions. The sensitivity scores between 1 week and 6 months were not different.[22] On the contrary, de Assis Cde et al., reported bacterial contamination and unsuccessful results after the application of GD to occlude the dentinal tubule.

Conventional treatment approaches for dentin hypersensitivity have a major drawback of being repeated frequently to achieve a continuous relief from pain as acidic diet or forceful toothbrushing results in continuing elimination of precipitates and surface coatings. Using lasers in the treatment of dentinal hypersensitivity could open new dimensions.[23]

Currently, different laser types with various settings and conditions are used for treating DH. The Nd:YAG laser (non-contact, 1 W, 100 mJ, 10 Hz, 60 s) was used in this investigation. The mechanism of Nd:YAG laser in the treatment of dentin hypersensitivity is believed to be the laser-produced blocking or narrowing of dentin tubules, in addition to direct nerve analgesia.[24]

SEM images in this study revealed that approximately all exposed dentin tubules in the Nd:YAG laser-treated specimens were entirely occluded, and a small number were partially occluded.

The findings of our study are similar to the finding of Abed et al. The ability of an Nd:YAG laser (1 W, 10 Hz, 60 seconds, non-contact mode without cooling) to seal exposed human dentinal tubules *in vitro* was compared with that of resin application. In comparison to the control group, laser treatment exhibited a more homogeneous dentinal surface with fewer exposed tubules and a 50% reduction in exposed tubule diameter.[24]

When Nd:YAG and diode (980 nm) lasers were tested alone or in combination with nano-bioglass, similar results to our study were found, that when used alone or in combination with the desensitizer, Nd:YAG laser significantly reduced the number of open dentinal tubules.[25] In another investigation, acidic beverages were applied to dentinal surfaces, which were subsequently treated with an Nd:YAG laser (0.6 W, 10 Hz, 60 mJ, and 85 mJ/cm^2) before being exposed to acidic beverages once more. The results demonstrated that irradiation with the Nd:YAG laser resulted in obliteration of dentinal tubules and reduction in their number, therefore altering the original structure.[26] Meanwhile, the occluding efficacy of 0.5 W power Diode, Nd:YAG and Er:YAG Lasers, applied over one layer of fluoride varnish, were found equally effective compared with the control group.[27] Nd:YAG laser was also found to be superior to CO_2 laser and 810 nm diode laser in reduction of dentinal tubule diameter, and less destructive compared with CO_2 laser, which had the maximum adverse effects of crater formation, cracks and charring of the surface.[28]

In our study, the combination of GD with Nd:YAG laser-treated specimens showed similar results to that of laser treatment alone as shown in SEM images in Figures 1D and E. Our findings were different from the findings reported by Lopes and Aranha,[29] with different dentin hypersensitivity protocols, Nd: YAG Laser (contact mode, 1.5 W, 10 Hz, and 100 mJ). GD agent, and its association were evaluated for 6 months in a randomized longitudinal clinical trial. They concluded that after 6 months of treatment, all therapies were useful in decreasing DH, but the combination of GD and Nd:YAG laser was an efficient therapeutic approach with immediate and long-lasting results. Farmakis et al.,[30] concluded that using 1 W Nd:YAG laser irradiation, alone or in combination with bioglass (NovaMin®), is a greater way to produce dentinal orifice occlusion and could lead to an efficient therapy for cervical dentin hypersensitivity. He attributed this effect to the process of "melting and re-solidification." Furthermore, the laser energy has been found to dissolve surface dentin, resulting in a sealing depth of approximately 4 µm within the tubules and leading to a prompt decrease in dentin hypersensitivity.[31,32]

Hydroxyapatite crystals contain Ca and P which are the primary inorganic constituents of dental hard tissue. According to previous research, certain chemical agents or treatments can affect the chemical structure of dentin, resulting in modifications to the Ca/P ratio. Changes in this ratio can impact the organic and inorganic components' relative proportions, which, in turn, can alter dentin permeability and solubility, and affect dental material adhesion.[10] However, our findings indicate that the Ca/P ratios were similar across all groups, and no significant differences were observed.

There are certain limitations to this study that should be mentioned. First, all the treatment modalities were used at one time, and it might require numerous applications to enhance its efficiency. Second, in an *in vitro* study to verify the interaction of these treatments with natural saliva is hard. So well-controlled clinical assessments are needed to ascertain the clinical efficacies of treatments used in this study. Third, one setting of parameters was used in this study; results may vary with different settings.

Conclusion

The GD was found to be effective in occluding the dentinal tubules compared with the control group. However, Nd:YAG laser and combination treatments were found to be more effective than the GD in blocking the dentinal tubules. Moreover, there was no difference in the occluding effect between Nd:YAG laser only and the combination treatments. The different tested treatment options did not significantly affect the mineral contents of the dentin.

Orcid

Hatem M El-Damanhoury https://orcid.org/0000-0003-4419-7872
Soumya S Aravind https://orcid.org/0000-0002-1947-253X
Betul Rahman https://orcid.org/0000-0002-8762-4187

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