

The Effects of Altering Er:YAG Laser Energy and Exposure Time on Exposed Dentinal Tubules: An In Vitro Study

Dina Hassouna¹ and Dahlia Ghazy Mohamed Rateb²

Abstract

Background: Different parameters of Erbium:Yttrium-Aluminum Garnet (Er:YAG) laser were considered in treatment of dentin hypersensitivity in previous clinical and in vitro studies.

Aim: To investigate the morphological aspects of inversely altering energy level and exposure time of Er:YAG laser on exposed dentinal tubules (DTs) in-vitro.

Methodology: Ten human freshly extracted sound third molars were collected. Their crowns were horizontally then vertically sectioned to expose transverse and longitudinal sections of DTs respectively. The obtained 20 specimens were divided into four groups (n=5) after exposure to 35% phosphoric acid: Group LI (irradiated with Er:YAG laser of 80 mJ energy and 60 seconds exposure time), group LII (irradiated with Er:YAG laser of 120 mJ energy and 30 seconds exposure time). Control groups (CI and CII) were not irradiated. Specimens were assessed by scanning electron microscope (SEM) provided with digitizer and the depth of laser impact was measured in irradiated groups for statistical analysis.

Results: Increasing energy and decreasing exposure time of Er:YAG laser in group LII adequately occluded exposed DTs, but the depth of laser impact was significantly reduced compared to group LI. Moreover, in group LII, the morphology of pulpal end of the DTs was adversely affected

Conclusion: Er:YAG laser parameters set of (80 mJ, 5 Hz, 60 s), under water spray was effective in occluding exposed dentinal tubules and harmless to deep dentin tissues which could provide a safe dentin hypersensitivity treatment that further needs to be assessed clinically.

Keywords: Er:YAG laser, Dentin hypersensitivity, Er:YAG laser energy level, Er:YAG laser time of exposure, Scanning electron microscopy and Digitizer.

1 Lecturer of Oral Biology Fayoum University, Cairo Egypt

2 Associate Professor of Oral Biology ,Faculty of Dentistry ,Ain Shams University,Cairo ,Egypt

Introduction:

Dentin hypersensitivity (DH) is one of the most encountered non-carious diseases nowadays due to changes in the lifestyle of the population, it was reported to affect one in three adults. Although being a frequent encountered complaint in clinical dental practice, it also one of the least successfully resolved dental problems ^{1,2,3,4}.

Dentin hypersensitivity defines a specific pain condition originating from exposed dentin with opened patent dentinal tubules (DTs) ⁵. This pain described as sharp, non-spontaneous, short or long-lasting originates from exposure of the dentin to thermal, chemical, mechanical, tactile, evaporative or osmotic stimuli, it cannot be attributed to any other dental pathology ^{6,7}. Hypersensitivity is a problem which makes the way of life difficult, it lowers general wellbeing, dictates food choices, affects social life and even the patient's overall self-esteem ^{8,9}.

The major contributing factors for dentin exposure and sensitivity are: loss of enamel and/or denudation of cervical root surface by loss of overlying cementum¹⁰, these factors come in consequence of many processes as abrasion, attrition, erosion, abfraction, gingival recession and periodontal treatment. Dental bleaching, root exposure with aging and improper brushing habits were also found to be potential causes of DH ^{10,11,12}. Individuals affected by DH encounter varying degrees of disturbance during eating, drinking and even breathing ¹³.

The currently most accepted theory explaining dental sensitivity ^{14,15,16} is the hydrodynamic theory proposed by Brännström ¹⁷. This theory postulates that when a stimulus is applied to opened tubules of dentin (due to non-carious lesions), it

causes a rapid shift of fluids within the DTs resulting in mechanical deformation of sensory nerves; present in the inner/ pulpal end of the tubules and responsible for pain production.

West ¹⁸ described the DTs' diameters to be significantly larger in a hypersensitive area compared to non-sensitive one. Sealing of DTs exposed to the oral environment by application of desensitizing agents which block the contact between tubules and external stimuli was the traditional strategy for management of DH ^{19,20,21}.

For a DH treatment to be effective, desensitizing agents must resist acid challenges and mechanical impediments encountered in the oral cavity ²². However, many of these agents were not found to have a long-term effect and the use of lasers for DH treatment became currently an efficient alternative, since proved to have an interesting long-term effect ²³.

Er:YAG laser gained popularity in the treatment of dental hard tissues after approved by Food and Drug Administration (FDA) in 1997 ²⁴. Er:YAG laser was used for the first time for DH therapy by Schwarz et al. ²⁵, where an immediate laser desensitization from a single session was reported to be effective and to maintain a more prolonged positive result compared with conventional desensitizing agents.

Some studies investigated the efficacy of Er:YAG laser in clinical management of painful hypersensitive teeth ^{26,25,27,28,29} in a trial to establish the optimal parameters of Er:YAG laser desensitization.

Therefore, the objective of this in vitro study was to explore the morphological effects of Er:YAG laser parameters suggested for tooth desensitization, on exposed DTs. The effect of inversely altering

energy level and exposure time of Er:YAG laser also investigated.

Material and Methods:

This study was approved by the Ethics Committee of the Faculty of Dentistry-Ain Shams University Approval No. (FDASU-Rec E092105).

Specimens preparation and study groups

Ten human freshly extracted, sound third molars teeth were used in this study. Teeth were collected from the Department of Surgery-Faculty of Dentistry- Ain-Shams University and only those free from caries, cracks, enamel and dentin pathology and restorations were selected. They were washed, cleaned and stored in 0.1% thymol solution (anti-microbial agent) for five days then stored until used in distilled water at 4°C for a maximum of one month. The teeth were individually mounted in autopolymerizing acrylic resin (Acrostone self-cure) with their roots embedded to cemento-enamel junction, thus exposing only coronal part of the teeth. To remove overlying enamel and expose superficial dentin surface, two millimeters were sectioned parallel to teeth occlusal surface³⁰ with a microsaw, under water coolant, leaving a remaining dentin thickness of 2.9 mm average as measured by caliper from the roof of the pulpal surface. Each crown was then cut mesio-distally into two halves; buccal and lingual hemicrowns; each presenting transverse and longitudinal sections of DTs at its occlusal and vertical cut surfaces, respectively.

To mimic opened DTs of hypersensitive dentin and remove the smear plugs, exposed dentin surfaces of all samples were subjected to application of 35% phosphoric acid (El-Gomhouria. Co, Egypt) for one min. The phosphoric acid was removed by rinsing with saline buffer for 40 s²⁹⁻³¹.

Sample size calculation

The sample size was estimated based on data from a previous study³² investigating the effect of Er:YAG laser on the depth of DTs' obliteration. The calculated effect size was 2.03, assuming an alpha error 0.05 and power 80% beta error 0.2, a sample of 4 cases per group was required. A sample of five cases per group for anticipated missing data was considered throughout the study. G*Power software for power analysis version 3.0.10 was used.

The segmented hemicrowns of this study were assigned into four groups (n= 5): two groups irradiated with different parameters of Er:YAG laser (LI and LII) and two control groups (CI and CII) that received no further treatment after exposure to 35% phosphoric acid. Due to variation in morphology of the DTs from tooth to another, the same tooth was used as experimental and control unit³¹.

Groups were divided as follows:

Group LI: buccal hemicrowns irradiated with Er:YAG laser of 80 mJ energy and 60 s exposure time.

Group LII: buccal hemicrowns irradiated with Er:YAG laser of 120 mJ energy and 30 s exposure time.

Control groups CI and CII: lingual hemicrowns not exposed to Er:YAG laser, where CI and CII corresponded respectively to irradiated groups LI and LII .

The samples were stored in distilled water until scanning electron microscopic evaluation.

Er:YAG laser treatment

Er:YAG laser device (Fotona 1210, Medical laser, Ljubljana, Slovenia) of 2940 nm wavelength was used for laser treatment.

The two irradiated groups LI and LII received Er:YAG laser of 5 Hz frequency, under a water spray with continuous and contact modes. The laser tip of the device was perpendicularly held at irradiation spot (occlusal side of the hemicrowns, in the

middle of its vertical cut edge). These conditions remained the same for the two irradiated groups, only energy and time of exposure parameters differed.

Scanning electron microscopic (SEM) examination

Amary 1810 SEM provided by IXRF 550i digitizer system (U.S.A) was used (National Center of Research, Cairo). All specimens were air-dried then sputter coated with gold for SEM examination of transverse and longitudinal sections of exposed DTs. In longitudinal sections, DTs' morphology was examined at the dentin subsurface and at the most deep end of the tubules adjacent to pulp. SEM images were captured at magnification 800x, 2000x, 100x. This was followed by inspection of irradiated groups LI and LII at low magnification 35x to detect the depth of laser impact into dentin with SEM digitizer and to investigate the morphology of the underlying dentin. For SEM digitizer analysis, measurements of axial depth of laser impact into dentin were tabulated in mm and mean was calculated for statistical analysis.

Statistical analysis: Data relative to the irradiated groups were performed by SPSS (version 20). Quantitative variables were described by the Mean, Standard Deviation (SD), the Range (Minimum – Maximum), Standard Error (SE) and 95% confidence interval of the mean. Shapiro-Wilk test of normality was used. Independent samples t-test was applied to compare the means of the two irradiated groups. Significance level considered at $P < 0.05$.

Results

Morphological observations

Untreated control group CI and CII showed the same results and were referred to as control group.

Some terminologies related to laser/dentin photothermal and thermomechanical tissue

interactions were used in irradiated groups LI and LII:

- Dentin melting: alteration in dentin surface caused by laser photothermal effect where dentin hydroxyapatite crystals melt and resolidify upon cooling taking various forms (eg melting beads and rugosities..)
- Cuff appearance: protrusion of peritubular dentin encircling opened DTs resulting from difference in removal of these two tissues by laser
- Carbonization (burning): color changes of tooth surface indicative of laser thermal damage.
- Dentin ablation: dentin removal upon laser irradiation where water and organic components of dental hard tissues readily absorb the high energy of laser and evaporate provoking micro explosions resulting in mechanical disruption of the tissue (thermomechanical interaction).

SEM results of transverse section in DTs

Untreated control group showed a smooth dentin surface topography, patent DTs and some scattered debris on the surface (fig. 1A). None of the lased dentin groups showed signs of cracking or carbonization. In lased group LI (80 mJ, 60 s), a rough, melted dentin surface with minute protrusions interspersed with few larger ones was observed. However, some opened DTs were detected and areas of dentin with apparently reduced-diameter were also seen (fig. 1B). Lased dentin of group LII (120 mJ, 30 s) showed a rough surface with irregular areas of rugosities and crater-like structures. Other areas revealed discrete beads of melted dentin blocking DTs and some scattered opened tubules with peritubular dentin appearing to be pulled out

than intertubular dentin giving a cuff-like appearance (fig. 1C).

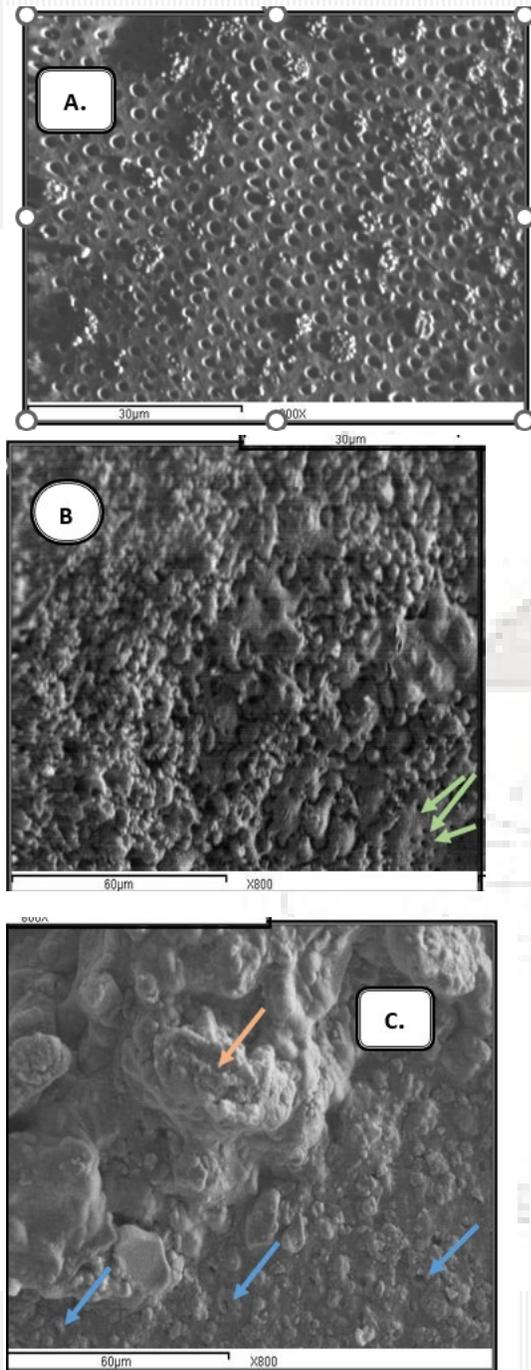


Fig. (1): Scanning electron micrographs of transverse section in dentinal tubules of control (A), LI (B) and LII (C) groups showing patent dentinal tubules (A), minute protrusions of melted dentin interspersed with few larger ones and an area of reduced-diameter dentinal tubules (green arrow) are shown in (B). Note crater-like structure (orange arrow), cuff-like appearance of peritubular dentin blue arrow and melted dentin beads in (C).

SEM results of longitudinal section in DTs at the subsurface

Beneath the occlusal exposed dentin surface, the control groups showed longitudinal profiles of patent DTs with opening of some canaliculi while others housed the lateral branches of odontoblastic processes (fig. 2 A). Both irradiated groups LI and LII showed an uneven, rough melted dentin surface with no visible signs of DTs (fig. 2 B&C)

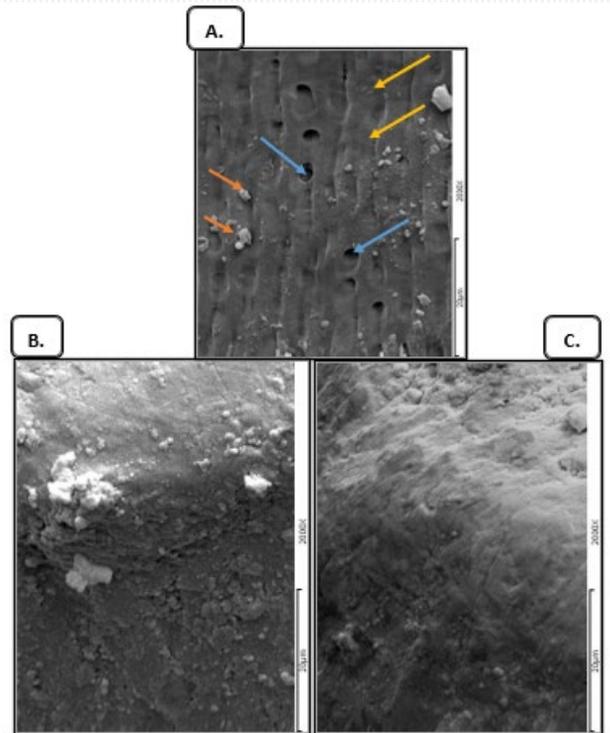


Fig. (2): Scanning electron micrographs of longitudinal section in dentinal tubules at the subsurface of control (A), LI (B) and LII (C) groups showing tubules profiles (yellow arrows) with patent canaliculi (blue arrows) and some lateral extensions of odontoblastic processes (orange arrows) (A). Groups LI (B) and LII (C) show a rough, melted dentin surface. 2000 x

SEM results of longitudinal section of DTs adjacent to pulp

The control groups showed DTs of normal architecture near the pulp (fig. 3A).

Irradiated group LI architecture was almost similar to the control fig. 3B. Group LII showed distinct beads of melted dentin in the zone adjacent to the pulp (fig. 3C).

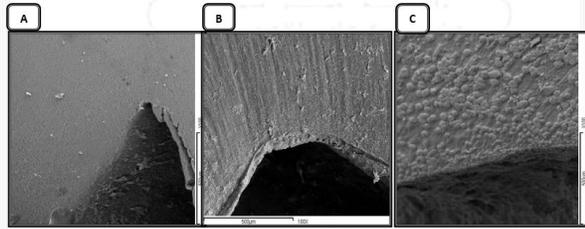


Fig. (3): Scanning electron micrographs of longitudinal section of DTs at the pulpal surface in control (A), LI (B) and LII (C) groups 100x

Results of SEM Digitizer and morphology of deep dentin in irradiated groups

Examination of representative SEM Digitizer images of irradiated groups showed laser impact in dentin subsurface represented by a whitish homogenous zone of melted dentin. In group LI, deeper dentin showed DTs of normal longitudinal architecture. The depth of laser impact in dentin subsurface of group LII was apparently shallower (both axially and laterally) than group LI, deeper dentin morphology revealed an irregular surface of elevated bands with absence of normal DTs architecture.

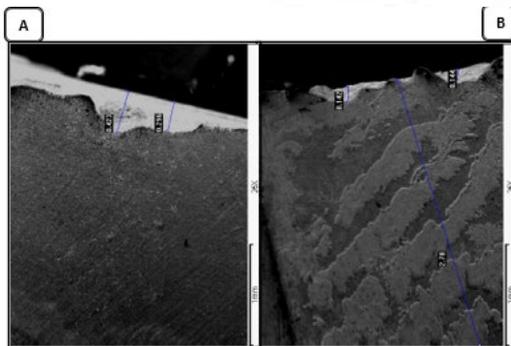


Fig. (4): Scanning electron micrographs of longitudinal section in dentin of the irradiated groups showing depth of laser impact with measurements of SEM digitizer and morphology of deeper dentin surface in groups LI (A) and LII (B).

Statistical analysis

Irradiated group LI showed a significant ($P < 0.05$) increase in axial depth of DTs

melting when compared with group LII table (1), fig. (5)

Table (1): Statistical results of axial depth (mm) of subsurface dentin melting in groups LI and LII

Group n=5	Mean	SD	Mean Differen ce	SE Differen ce	95% Confidence Interval of the Difference		P Value
					Lower	Upper	
Group LI	0.56	0.19	0.41	0.10	0.18	0.63	0.0030 9*
Group LII	0.15	0.10					

* Significant $P < 0.05$

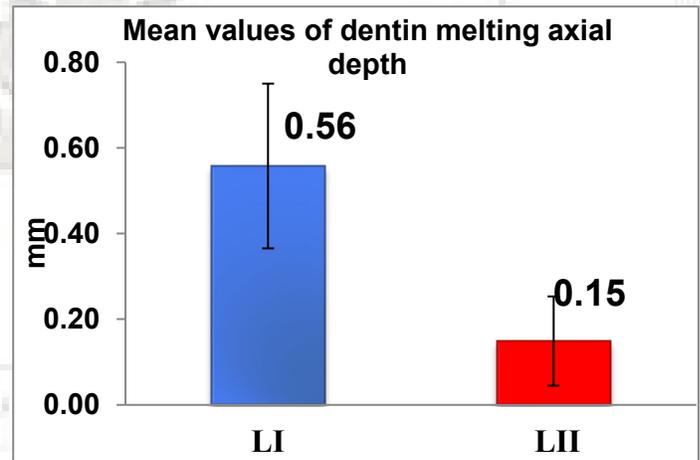


Fig. (5): Bar chart illustrating mean values of axial depth of dentin subsurface melting (mm) of irradiated groups LI and LII

Discussion

Er:YAG laser was considered by a variety of researchers to be the recommended laser type for treatment of DH due to its superior absorption properties within hard dental tissue, as well as its pulp safety over other types 33–35.

The clinical efficacy of Er:YAG laser was previously reported to reduce sensitivity of exposed hypersensitive dentin 25,27,29,36–38, the improvement rate documented by Yu and Chang 38 was as high as 90%. In the current study, the selected energy levels

were comparable to low energy levels previously used in clinical studies for tooth desensitization 27,38,39.

When using Er:YAG laser to treat DH in dental practice, parameters vary between device brands due to differences in setups. Optimal parameters of Er:YAG laser for desensitization treatment are usually low 30. However, low level energy is not set in all devices. Therefore, this study aimed to test the effect of inversely altering energy level of Er:YAG laser and its time of exposure (by increasing energy level and decreasing time of exposure) on the morphology of exposed DTs.

Considering that 80 mJ Er:YAG (a previously used energy level in desensitization studies) 25,38,39 was the lowest set energy level used in this study, it represented the starting value for parameters exploration with an exposure time of 60 s. Knowing that high laser energy could be harmful to dental hard tissues and pulp 40, the selected energy level and exposure time of the other irradiated group in this study were 120 mJ and 30 s respectively.

In the present study, to widely open DTs simulating dentin hypersensitivity models, one min application of 35% phosphoric acid (30, 31) was used on exposed dentin surfaces. SEM micrographs of transverse sections of control group showed a smooth dentin surface, free of smear layer, with wide-opened DTs which is in agreement with several studies 29–31,41,42. In longitudinal sections of the same group, longitudinal profiles of DTs were seen with some sporadically projecting lateral branches of the odontoblastic processes, which was in accordance with other studies 43–45.

Examination of scanning electron micrographs of transverse sections of DTs of group LI revealed a surface with minute protrusions of melted dentin surface interspersed with few larger ones and some

areas of reduced- diameter DTs. These results were parallel to other studies 29,31 revealing that irradiation of low level intensity Er: YAG laser (60 mJ for 60 s) used for desensitization purpose, caused partial obliteration of exposed DTs in the irradiated spot as well as signs of dentin melting that masked the DTs. Our findings also came along with those of a study 30 exploring the occluding effects of Er:YAG laser (50 mJ energy and 30s exposure time) on DTs where melted dentin with bubbles appearance and few partially occluded DTs were reported.

Meanwhile, SEM results of group LI were not similar to those of Cakar et al.46 showing appearance of craters on dentin surface irradiated with Er:YAG laser of 60 mJ for 10 sec, at 30 Hz frequency. This discrepancies in results might be attributed to different laser frequency used. The frequency was 30 Hz in Cakar's study, whereas 5 Hz was used in the present study. The investigators described the DT orifices to be obviously occluded but depressed, giving crater appearance.

One the most approved theories trying to explain the effect of high power output lasers; including Er:YAG, on dentin pointed out to sealing of DTs by dentin melting and re-crystallization47. It was earlier explained that thermal energy generated by laser was quickly absorbed by dentin, melting its hydroxyapatite structure which upon cooling, re-solidified forming larger hydroxyapatite crystals than existed in the initial structure. By recrystallization of dentin, a nonporous surface was produced with partially or totally obliterated DTs 48. It was further clarified that melting and fusion of the dentin hard surface layer was a favorable effect of high power lasers; attributed to their photothermal mechanism and consequently occluding opened DTs. The dentin hydraulic conductance was thus

decreased with a consequent reduction of dentin sensitivity 37,49,50.

In addition to occlusion of exposed DTs by partial melting after low-intensity Er:YAG irradiation and reduction of the effect of external stimulation on dental pulp 46, an analgesic effect on pulpal nerves was implicated in the process of instant desensitization 51. The decreased fluid movement within DTs upon irradiation with Er:YAG laser was also attributed to evaporation of the superficial layers of the dentinal fluid caused by high absorption of Er:YAG laser emission wavelength in water 38.

In the present study, scanning electron micrographs of DTs transverse section of group LII (120 mJ, 30 seconds) revealed an uneven melted, rough surface with crater-like structures, occluded DTs with beads of dentin melting and some others opened tubules. The opened DTs showed protrusion of peritubular dentin appearing to be pulled out and giving cuff-like appearance.

The cuff-like appearance of protruded peritubular dentin encircling opened DTs was similarly observed in several studies 52–54 with various parameters of Er:YAG laser exposure. It was explained that rate of removal of intertubular dentin was more than peritubular dentin and that this selective dentin tissue removal was attributed to the different water content of these dentin structures. Intertubular dentin tissues containing more water are further removed than peritubular dentin containing reduced water amount. Protrusion of peritubular dentin from the surface was also reported by Wang et al. 55 when dentin was irradiated with Er:YAG parameters of 100mj energy level, for 10 s, at 5Hz frequency, which are parameters comparable to those used in group LII.

On the other hand, dentin ablation (removal) was reported in Nahas' study 56 upon irradiating dentin with Er:YAG laser of

120mj at 10 Hz frequency for 60 s with air/water coolant and 5 μ s pulsed mode. Despite the same energy level used in the two studies, we cannot compare our results with those of Nahas's due to variations of other parameters namely, frequency, exposure time, cooling system (air/water) and laser operating mode.

In the current study, it could be observed from the obtained results that the parameters used in both Er:YAG irradiated groups modified surface morphology of DTs when compared with the control. However, it cannot be affirmed from surface results that parameters of a specific group were more aggressive than the other, since a single morphological pattern was not established for each group, but rather an involvement of diverse patterns. Though, it could be concluded that the parameters used in both irradiated groups seem to be suitable for occlusion of DTs.

Many studies focused on investigating the surface of Er:YAG lased dentin, but a limited number of studies examined longitudinal sections of lased dentin. This is probably due to the fact that available conventional dentin sample preparation methods (dentin disc samples), which are developed for surface investigations do not allow deep dentin layers examination 57, 58. In this study, examination of scanning electron micrographs of dentin subsurface in irradiated groups LI and LII revealed signs of melting in dentin subsurface with masking of longitudinal architecture of DTs, which is consistent with the scanning electron micrographs of their DTs' transverse section.

The most critical issue in any laser therapy is to determine the correct parameters to achieve satisfactory results without thermal detrimental pulpal effects, fractures and carbonization 31,59,60, so that maximum laser efficiency could be attained with least heat effect or damage of dental hard tissues

56. So, considering that Er:YAG laser gave satisfactory DTs obliteration results without fractures and carbonization as revealed from surface and subsurface results of the two irradiated groups LI and LII, investigating the morphology of deeper dentinal tissues was necessary.

In the current study, examination of longitudinal sections of DTs near the pulp in group LI revealed a DT topography almost similar to that of the control group. This finding indicates that the Er:YAG parameters (80mJ - 5Hz for 60 seconds in continuous mode) can be considered safe to pulp. Meanwhile, increasing Er:YAG energy and shortening its exposure time in group LII revealed melting beads at the inner end of DTs with obvious masking of tubules' architecture. The results of group LII are in parallel with those of a study 32 reporting a well-defined melting area masking the DTs' architecture at their most inner zone, upon irradiating exposed dentin with Er:YAG of 200 mJ and 30 s exposure time.

It is known that alterations taking place in hard dental tissues following laser irradiation are mainly caused by photothermally-induced temperature rise in the tissue 34, 35. Pulpal safety was not considered a significant concern when using Er:YAG laser because of its negligible depth of energy penetration 61–64.

Although, it could be suggested from the melting beads observed at the most inner side of DTs, in longitudinal section of group LII, that pulp could be affected upon increasing energy of Er:YAG to 120 mJ and decreasing its exposure time to 30 s. Further studies for intrapulpal temperature measurements and pulp tissue morphology could be required to investigate pulp condition with these suggested parameters. In the present study, surface scanning electron micrographs of both Er:YAG lased groups (LI, LII) revealed absence of surface cracking or carbonization after irradiation

indicating absence of undesirable thermal damage as pointed out by several studies 65–67. Nevertheless, presence of melting beads in SEM images of longitudinal section of DTs of group LII near the pulp may support the contention that thermal effects of Er:YAG laser irradiation reached the deepest dentin area and promoted the observed melting changes. This was further supported by low magnification photomicrographs of group LII where bands of elevated irregular dentin were seen underneath the subsurface melted dentin. The results of group LII are also parallel with those of Kilinc et al. 63 where Er:YAG laser was reported to produce less heat on irradiated surface than on pulpal wall. It was previously reported that, when used on hard dental tissues, the wavelength of Er:YAG laser penetrates approximately 5 micrometers in dentin and ideally, the remaining dental tissue should not be affected, thereby allowing minimal damage to the surrounding tissue 68. The same investigators also added that spread out of absorbed laser energy into surrounding tissue (indirect tissue heating) occurring by a process of thermal diffusion leads to undesirable thermal effects and therefore must be avoided. In longitudinal sections of group LII of the herein study, appearance of melting beads in dentin near pulp suggested that laser parameters set (120 mJ, 30 sec, 5Hz) can cause spread out of irradiated Er:YAG laser energy into deeper dentin.

Due to scarcity of studies conducted to examine the dentin underlying Er:YAG lased, exposed dentin surface 67, longitudinal sections of the irradiated groups were inspected at low magnification with SEM Digitizer to investigate the depth of Er:YAG laser impact in dentin subsurface. Morphology of underlying dentin was also examined.

Statistical results of SEM Digitizer revealed that depth of laser dentin melting in group

LI was significantly increased compared to group LII which also points out that obliteration depth of DTs in group LI was greater than group LII. Variation in obliteration depth of the DTs observed in the two irradiated Er:YAG groups is consonant with the results of several studies 31,32,69,70 indicating that diverse parameters of Er:YAG laser below ablation threshold could be a promising treatment for reducing permeability through exposed dentinal tubules, but to varying degrees and depths.

After examining morphology of DTs underneath the irradiated area in the three longitudinal sections of the irradiated groups of this study, it could be noted that: in irradiated group LI, directly beneath the melted dentin subsurface, DTs were almost intact till reaching the pulp cavity. Whereas in irradiated group LII, DTs were directly affected beneath the melted dentin subsurface till their most inner/pulpal end where melted beads were observed. It was surprisingly noted that though the depth of laser impact in group LII was significantly decreased compared to group LI, yet deep dentin of group LII was adversely affected which could also have a detrimental effect on pulp. This study provides useful information on the choice of appropriate parameters to use during application of Er:YAG laser for desensitization purposes.

Conclusion:

Based on the morphological results of this in vitro study, it could be concluded that Er:YAG laser parameters set of (80 mJ, 5 Hz, 60 s), under a water spray was effective in occluding exposed dentinal tubules and harmless to deeper dentin tissues, which provided the theoretical basis for safe dentin hypersensitivity treatment.

- When increasing energy and decreasing exposure time (120 mJ, 30 s) of

Er:YAG laser under the same other working conditions, exposed dentinal tubules were adequately occluded, but depth of laser impact was significantly reduced compared to (80 mJ, 60 s) parameters. Moreover, Er:YAG parameters (120 mJ, 30 s) adversely affected the morphology of pulpal end of the dentinal tubules which could be detrimental to dental pulp.

Recommendation

- Further studies are recommended to investigate safety of suggested parameters of this study on dental pulp.
- Studies simulating intraoral conditions, with brushing and acidic challenges are required to investigate whether suggested parameters of this study give long-lasting results in treating dentin hypersensitivity.
- A clinical study using the suggested parameters should be conducted for safe and effective treatment optimization.

References:

1. Banfield N, Addy M. Dentine hypersensitivity: Development and evaluation of a model in situ to study tubule patency. *J Clin Periodontol.* 2004;31(5):325–35.
2. Rösing CK, Fiorini T, Liberman DN, Cavagni J. Dentine hypersensitivity: Analysis of self-care products. *Braz Oral Res.* 2009;23(SUPPLEMENT 1):56–63.
3. Aparna S, Setty S, Thakur S. Comparative efficacy of two treatment modalities for dentinal hypersensitivity: a clinical trial. *Indian J Dent Res.* 2010;21(4):544–8.
4. Zeola LF, Soares PV, Cunha-Cruz J. Prevalence of dentin hypersensitivity: Systematic review and meta-analysis. *J Dent.* 2019;81:1–6.
5. Cohen , Hargreaves, Kenneth M., S. Pathways of the pulp [Internet]. St. Louis, Mo.: Mosby Elsevier; 2006.
6. Holland GR. Guidelines for the design and conduct of clinical trials on dentine hypersensitivity. *J Clin Periodontol.* 1997;24(11):808–13.
7. Orchardson R, Gillam DG. Practical Science. *J Am Dent Assoc.* 2006;137(7):990–8.

8. Mason S, Burnett GR, Patel N, Patil A, Maclure R. Impact of toothpaste on oral health-related quality of life in people with dentine hypersensitivity. *BMC Oral Health* [Internet]. 2019;19(1):226.
9. Mazur M, Jedliński M, Ndokaj A, Ardan R, Janiszewska-olszowska J, Nardi GM, et al. Long-term effectiveness of treating dentin hypersensitivity with bifluorid 10 and futurabond u: A split-mouth randomized double-blind clinical trial. *J Clin Med*. 2021;10(10).
10. Trushkowsky RD, Oquendo A. Treatment of Dentin Hypersensitivity. *Dent Clin North Am* [Internet]. 2011;55(3):599–608.
11. Roberson Heymann, Harald., Swift, Edward J., Sturdevant, Clifford M., TM. Sturdevant's art and science of operative dentistry. Edinburgh: Elsevier Mosby; 2006.
12. Porto ICCM, Andrade AKM, Montes MAJR. Diagnosis and treatment of dentinal hypersensitivity. *J Oral Sci*. 2009;51(3):323–32.
13. Splieth CH, Tachou A. Epidemiology of dentin hypersensitivity. *Clin Oral Investig* [Internet]. 2013;17(1):3–8.
14. Dababneh RH, Khouri AT, Addy M. Dentine hypersensitivity—An enigma? A review of terminology, mechanisms, aetiology and management. *Br Dent J*. 1999;187(11):606–11.
15. Addy M. Dentine hypersensitivity: new perspectives on an old problem. *Int Dent J*. 2002;52(S5P2):367–75.
16. Cartwright RB. Dentinal hypersensitivity: a narrative review. *Community Dent Heal*. 2014;31(1):15–20.
17. Brännström M. Sensitivity of dentine. *Oral Surgery, Oral Med Oral Pathol*. 1966;21(4):517–26.
18. West NX, Lussi A, Seong J, Hellwig E. Dentin hypersensitivity: Pain mechanisms and aetiology of exposed cervical dentin. *Clin Oral Investig*. 2013;17(SUPPL.1):9–19.
19. Gillam DG, Newman HN, Davies EH, Bulman JS, Troullos ES, Curro FA. Clinical evaluation of ferric oxalate in relieving dentine hypersensitivity. *J Oral Rehabil*. 2004;31(3):245–50.
20. Tengrungsun T, Sangkla W. Comparative study in desensitizing efficacy using the GaAlAs laser and dentin bonding agent. *J Dent*. 2008;36(6):392–5.
21. Hoang-Dao B, Hoang-Tu H, TRAN-THI N, Koubi G, Camps J, About I. Clinical efficiency of a natural resin fluoride varnish (Shellac F) in reducing dentin hypersensitivity. *J Oral Rehabil*. 2009;36(2):124–31.
22. Han SY, Kim JS, Kim YS, Kwon HK, Kim B II. Effect of a new combined therapy with nano-carbonate apatite and co2 laser on dentin hypersensitivity in an in situ model. *Photomed Laser Surg*. 2014;32(7):394–400.
23. Hu M-L, Zheng G, Han J-M, Yang M, Zhang Y-D, Lin H. Effect of lasers on dentine hypersensitivity: evidence from a meta-analysis. *J Evid Based Dent Pract*. 2019;19(2):115–30.
24. Cozean C, Arcoria CJ, Pelagalli J, Powell GL. Dentistry for the 21st century? Erbium: YAG laser for teeth. *J Am Dent Assoc*. 1997;128(8):1080–7.
25. Schwarz F, Arweiler N, Georg T, Reich E. Desensitizing effects of an Er: YAG laser on hypersensitive dentine: a controlled, prospective clinical study. *J Clin Periodontol*. 2002;29(3):211–5.
26. Zhang C, Matsumoto K, Kimura Y, Harashima T, Takeda FH, Zhou H. Effects of CO2 laser in treatment of cervical dentinal hypersensitivity. *J Endod* [Internet]. 1998;24(9):595–7.
27. Birang R, Poursamimi J, Gutknecht N, Lampert F, Mir M. Comparative evaluation of the effects of Nd:YAG and Er:YAG laser in dentin hypersensitivity treatment. *Lasers Med Sci*. 2007;22(1):21–4.
28. Ipci SD, Cakar G, Kuru B, Yilmaz S. Clinical evaluation of lasers and sodium fluoride gel in the treatment of dentine hypersensitivity. *Photomed Laser Surg*. 2009;27(1):85–91.
29. Badran Z, Boutigny H, Struillou X, Baroth S, Laboux O, Soueidan A. Tooth desensitization with an Er: YAG laser: in vitro microscopical observation and a case report. *Lasers Med Sci*. 2011;26(1):139–42.
30. Zhuang H, Liang Y, Xiang S, Li H, Dai X, Zhao W. Dentinal tubule occlusion using Er: YAG Laser: an in vitro study. *J Appl Oral Sci*. 2021;29.
31. Aranha ACC, Domingues FB, Franco VO, Gutknecht N, Eduardo C de P. Effects of Er:YAG and Nd:YAG lasers on dentin permeability in root surfaces: a preliminary in vitro study. *Photomed Laser Surg*. 2005 Oct;23(5):504–8.
32. Hassouna DM, Girgis AS, Hakam HM, Gheith ME. In vitro study to evaluate the effect of Er: YAG laser with different parameters on exposed dentin in human teeth (scanning electron microscope and stereomicroscope assessments). *C D J*. 2013. 29(1):1-7.
33. Gouw-Soares S, Pelino JEP, Haypek P, Bachmann L, Eduardo C de P. Temperature rise in cavities prepared in vitro by Er: YAG laser. *J Oral Laser Appl*. 2001;1:119–23.
34. Parker S. Surgical lasers and hard dental tissue. *Br Dent J*. 2007;202(8):445–54.

35. Coluzzi DJ, Convissar RA. Principles and practice of laser dentistry. St Louis, Mo Mosby Elsevier. 2011;12–26.
36. Dilsiz A, Aydin T, Canakci V, Gungormus M. Clinical evaluation of Er: YAG, Nd: YAG, and diode laser therapy for desensitization of teeth with gingival recession. *Photomed Laser Surg.* 2010;28(S2):S-11-7.
37. Aranha ACC, de Paula Eduardo C. Effects of Er: YAG and Er, Cr: YSGG lasers on dentine hypersensitivity. Short-term clinical evaluation. *Lasers Med Sci.* 2012;27(4):813–8.
38. Yu CH, Chang YC. Clinical efficacy of the Er:YAG laser treatment on hypersensitive dentin. *J Formos Med Assoc [Internet].* 2014;113(6):388–91.
39. Oberhofer O, Sculean A. Er: YAG Laser and Desensitizing Effect on Dentin and Dental Cervices. *J Oral Laser Appl.* 2008;8(3).
40. Brandão CB, Contente MMMG, De Lima FA, Galo R, Corrêa-Afonso AM, Bachmann L, et al. Thermal alteration and morphological changes of sound and demineralized primary dentin after Er: YAG laser ablation. *Microsc Res Tech.* 2012;75(2):126–32.
41. Belal MH, Yassin A. A comparative evaluation of CO₂ and erbium-doped yttrium aluminium garnet laser therapy in the management of dentin hypersensitivity and assessment of mineral content. *J Periodontal Implant Sci.* 2014;44(5):227–34.
42. Öncü E, Karabekiroğlu S, Ünlü N. Effects of different desensitizers and lasers on dentine tubules: An in-vitro analysis. *Microsc Res Tech.* 2017;80(7):737–44.
43. Wang T, Yang S, Wang L, Feng H. Use of poly (amidoamine) dendrimer for dentinal tubule occlusion: A preliminary study. *PLoS One.* 2015;10(4) :e0124735.
44. Chiang YC, Wang YL, Lin PY, Chen YY, Chien CY, Lin HP, et al. A mesoporous biomaterial for biomimetic crystallization in dentinal tubules without impairing the bonding of a self-etch resin to dentin. *J Formos Med Assoc [Internet].* 2016;115(6):455–62.
45. A Aziz AA, El Imam HF. Microtensile bond strength and nanoleakage of dentin surfaces pretreated with different etching materials. *Egypt Dent J.* 2018;64(4-October (Fixed Prosthodontics, Dental Materials, Conservative Dentistry & Endodontics)):4003–12.
46. Cakar G, Kuru B, Ipci SD, Aksoy ZM, Okar I, Yilmaz S. Effect of Er: YAG and CO₂ lasers with and without sodium fluoride gel on dentinal tubules: a scanning electron microscope examination. *Photomed Laser Surg.* 2008;26(6):565–71.
47. Kimura Y, Wilder-Smith P, Yonaga K, Matsumoto K. Treatment of dentine hypersensitivity by lasers: a review. *J Clin Periodontol Rev Artic.* 2000;27(10):715–21.
48. Asnaashari M, Moeini M. Effectiveness of lasers in the treatment of dentin hypersensitivity. *J lasers Med Sci.* 2013;4(1):1-7.
49. Biagi R, Cossellu G, Sarcina M, Pizzamiglio IT, Farronato G. Laser-assisted treatment of dentinal hypersensitivity: a literature review. *Ann Stomatol (Roma).* 2015;6(3–4):75.
50. Rezazadeh F, Dehghanian P, Jafarpour D. Laser effects on the prevention and treatment of dentinal hypersensitivity: A systematic review. *J Lasers Med Sci [Internet].* 2019;10(1):1–11.
51. Zeredo JL, Sasaki KM, Fujiyama R, Okada Y, Toda K. Effects of low power Er: YAG laser on the tooth pulp-evoked jaw-opening reflex. *Lasers Surg Med Off J Am Soc Laser Med Surg.* 2003;33(3):169–72.
52. Delmé KIM, De Moor RJG. Scanning electron microscopic evaluation of enamel and dentin surfaces after Er: YAG laser preparation and laser conditioning. *Photomed Laser Surg.* 2007;25(5):393–401.
53. He Z, Otsuki M, Sadr A, Tagami J. Acid resistance of dentin after erbium: yttrium-aluminum-garnet laser irradiation. *Lasers Med Sci.* 2009;24(4):507–13.
54. Esteves-Oliveira M, de Guglielmi CAB, Ramalho KM, Arana-Chavez VE, de Eduardo CP. Comparison of dentin root canal permeability and morphology after irradiation with Nd: YAG, Er: YAG, and diode lasers. *Lasers Med Sci.* 2010;25(5):755–60.
55. Wang J hui, Yang K, Zhang B ze, Zhou Z fei, Wang Z rui, Ge X, et al. Effects of Er:YAG laser pre-treatment on dentin structure and bonding strength of primary teeth: an in vitro study. *BMC Oral Health [Internet].* 2020;20(1):1–10.
56. Nahas P, Zeinoun T, Namour M, Ayach T, Nammour S. Effect of er:Yag laser energy densities on thermally affected dentin layer: Morphological study. *Laser Ther.* 2018;27(2):91–7.
57. Apel C, Meister J, Götz H, Duschner H, Gutknecht N. Structural changes in human dental enamel after subablative erbium laser irradiation and its potential use for caries prevention. *Caries Res.* 2005;39(1):65–70.
58. Cvikl B, Lilaj B, Franz A, Degendorfer D, Moritz A. Evaluation of the morphological characteristics of laser-irradiated dentin. *Photomed Laser Surg.* 2015;33(10):504–8.

59. Lan WH, Liu HC. Treatment of dentin hypersensitivity by Nd:YAG laser. *J Clin Laser Med Surg.* 1996 Apr;14(2):89–92.
60. Liu H-C, Lin C-P, Lan W-H. Sealing depth of Nd:YAG laser on human dentinal tubules. *J Endod [Internet].* 1997;23(11):691–3.
61. Wigdor H, Abt E, Ashrafi S, Walsh JTJ. The effect of lasers on dental hard tissues. *J Am Dent Assoc.* 1993 Feb;124(2):65–70.
62. Geraldo-Martins VR, Tanji EY, Wetter NU, Nogueira RD, Eduardo CP. Intrapulpal temperature during preparation with the Er: YAG laser: an in vitro study. *Photomed Laser Ther.* 2005;23(2):182–6.
63. Kilinc E, Roshkind DM, Antonson SA, Antonson DE, Hardigan PC, Siegel SC, et al. Thermal safety of Er: YAG and Er, Cr: YSGG lasers in hard tissue removal. *Photomed Laser Surg.* 2009;27(4):565–70.
64. Raucci-Neto W, Raquel dos Santos C, Augusto de Lima F, Pécora JD, Bachmann L, Palma-Dibb RG. Thermal effects and morphological aspects of varying Er:YAG laser energy on demineralized dentin removal: an in vitro study. *Lasers Med Sci.* 2015;30(4):1231–6.
65. Staninec M, Meshkin N, Manesh SK, Ritchie RO, Fried D. Weakening of dentin from cracks resulting from laser irradiation. *Dent Mater.* 2009;25(4):520–5.
66. Kazmiruk BGE-V. Effects of Er:YAG Laser Irradiation on Dental Hard Tissues and All-Ceramic Materials: SEM Evaluation. In Rijeka: IntechOpen; 2012.
67. He Z, Chen L, Hu X, Shimada Y, Otsuki M, Tagami J, et al. Mechanical properties and molecular structure analysis of subsurface dentin after Er: YAG laser irradiation. *J Mech Behav Biomed Mater.* 2017;74:274–82.
68. Diaci J, Gaspirc B. REVIEW. Comparison of Er : YAG and Er , Cr : YSGG lasers used in dentistry Comparison of Er : YAG and Er , Cr : YSGG lasers used in dentistry. *J Laser Heal Acad.* 2012;2012(1):1–13.
69. Matsui S, Kozuka M, Takayama J, Ueda K, Nakamura H, Ito K, et al. Stimulatory Effects of CO(2) Laser, Er:YAG Laser and Ga-Al-As Laser on Exposed Dentinal Tubule Orifices. *J Clin Biochem Nutr.* 2008 Mar;42(2):138–43.
70. Kurt S, Kırtıloğlu T, Yılmaz NA, Ertaş E, Oruçoğlu H. Evaluation of the effects of Er:YAG laser, Nd:YAG laser, and two different desensitizers on dentin permeability: in vitro study. *Lasers Med Sci [Internet].* 2018;33(9):1883–90.