SEALING EFFECT OF DIODE LASER 940 NM ON THE DENTINAL TUBULES (IN VITRO STUDY)

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ABSTRACT
The aims of this study was to evaluate the efficacy of using diode laser (940nm) in the occlusion of dentinal tubules and the safe parameters to the dental tissues. Materials and methods: fifty premolar teeth were used. Dentin surface irradiated with diode laser 940 nm at different output power setting: 0.8, 1.6, 2, and 3 W, for 10 seconds in continuous and noncontact mode. Samples were divided into 5 groups: the control group, laser at 0.8, 1.6, 2 and 3W groups. Results: maximum temperature rise was 3°C. Diode laser irradiations at 1.6, 2 W led to effectively narrowing or sealing of dentinal tubules without provoking carbonization. Conclusions: Diode laser 940 nm used at 1.6, 2 W for 10 sec in continuous mode was able to seal the dentinal tubules. These parameters can be regarded harmless for dental tissues, and could be effective in the treatment of dentinal hypersensitivity.

KEYWORDS: Dentinal tubules, sealing, 940nm, diode laser, temperature.

INTRODUCTION
Dentin hypersensitivity (DH) is explained as a well-localized, sharp, short pain as a reaction to thermal, tactile, osmotic, or chemical stimuli which cannot be attributed to any other type of dental pathology (Trashkowsky and Oquendo 2011). It is referred to regard as a symptom complex, rather than a disease. It occurs as a result of exposed dentin (Cohen, Burns et al., 1998). The prevalence of dentin hypersensitivity is more than 40% of adults worldwide (Irwin and McCusker, 1996). The cervical area of teeth is the most affected site (Orchardson and Collins, 1987). Premolars are the most common teeth affected (Addy, 2000). There are many causes for dentin sensitivity. Enamel loss and cementum denudation are the major contributing factors (Trashkowsky and Oquendo 2011) and they occur as a result of: attrition, abrasion, erosion, abfraction, gingival recession, bleaching, periodontal treatment. Hypersensitive teeth have a larger number and wider diameter dentinal tubules (DTs) than those of nonsensitive teeth (Kerns, Scheidt et al., 1991). According to Brännström’s hydrodynamic theory (Brännström, 1963), the movement of fluid across the DTs stimulates the mechano-receptors in or near the pulp, the occlusion or narrowing the patent DTs reduces dentin permeability and, correspondingly, decreases the grade of DH (Rimondini, Baroni et al., 1995). Different desensitizing agents have been suggested to treat DH, but studies revealed that they are ineffective or not long lasting (Kishore, Mehrotra et al., 2002). Recently, the clinical practice of lasers had increased. For the management of DH, the mechanism of action differ with lasers, for (low-level) power lasers, they act directly on the pulp nerve endings causing analgesia by inducing change in neural transmission, while for (high-level) power lasers, they provoke melting with recrystallization in the dentine surface resulting in occlusion of DTs (Umberto, Claudia et al., 2012).

Several studies reported that Nd: YAG, CO2 (high-level) are useful in the occlusion of dentinal tubules (Pashley, Horner et al., 1992, Lan and Liu, 1995; Moritz Schoop et al., 1998, Maamary, De Moor et al., 2008). Diode laser was suggested to treat DH. Diode laser (low-level) can act directly by depressing neural transmission of stimuli. But long term success in clinical use is questionable (Sgolastra, Petruci et al., 2011), and in higher degrees of DH its efficacy seems poor. Few studies have reported that (high-level) diode laser 810 and 980nm can seal DTs (Umana, Heysselaer et al., 2013), and no study yet investigate the effect of (high-level) diode laser 940nm in the occlusion of DTs, like other types of (high-level) lasers. The aims of this study was to evaluate, under a scanning electron microscope (SEM), the efficacy of using diode laser (940nm), and the safe parameters to the dental tissues.

METHODOLOGY
Fifty adult human upper premolar teeth of two roots extracted for orthodontic purpose had been utilized after approval of the Ethics Review Committee of Baghdad University on research (Nr: 5033/15). Crowns with caries, restorations, or cracks were discarded. They were kept for two weeks in 4°C distilled water containing 0.1% thymol to hinder microbial growth until use. Teeth were mounted on a holder for stabilization, the cementum was removed by 70 times strokes as stated by Coldiron et al.,1990 using a periodontal curette, then teeth were immersed in 17% ethylenediaminetetraacetic acid (dental product SA Co., Switzerland) solution for 1 minute to remove smear layer, then rinsed with distilled water in ultrasonic bath for 15 minutes, and dried with gauze. An area of (3X4 mm), in the buccal surface of cervical third of the tooth, was marked to determine the lasing area. Ten teeth were prepared, two per group, for SEM analysis. To assess temperature and thickness measurement, forty premolars...
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were used, ten per group. In the lingual surface, opposite to the lasing area, a hole was made by diamond fissure bur until reach the pulp cavity to insert a thermocouple. For thickness assessment, the teeth were sectioned horizontally through the mid-length of the marked lasing area. Samples were divided into five groups, the control group which was received no treatment, 940 nm diode laser EPIC™ (BIOLASE, San Clement, CA, USA, power output: 10 Watt) at 0.8, 1.6, 2 and 3W groups. The used laser settings: continuous, noncontact mode 1 mm distance, exposure time 10 seconds, power densities 323.8, 647, 809.7, 1214.57 W/cm², energy densities 3238,6470,8097,12145.7 J/cm² for the following output power: 0.8, 1.6, 2, 3W respectively, laser fiber tip diameter: 300 µm which was held at 45 degree angle to the irradiated surface, spot size diameter: 0.561µm J ust one session for each sample.

Temperature Measurement
Temperature assessment was done by immersion part of the root in water, which was heated by a waterbath (BS-11, Korea) to ensure 37 ± 0.5°C steady teeth temperature at. A K type thermocouple was utilized (Amprobe TMD-56, Everett, WA, USA), which had an accuracy of 0.05°C. The temperature was recorded every second. A thermo conductor grease of 1W/MK thermal conductivity (Hutixi Co., China) was injected inside the pulp chamber, to ensure maximal thermal conduction between thermocouple probe and the inner dentin. Then teeth were sectioned horizontally through the marked lasing area to assess the dentin thickness by a Vernier caliper (TOPEX Sp. S.K., Warsaw, Poland).

SEM analysis
Samples fixation and dehydration, in an ascending ethanol series, were done following the protocol used by (Marchesan, Brugnera-Junior et al., 2008). After dehydration, the specimens were left to dry for 24h, then fixed on aluminum stubs and metallized with a layer of gold, using vacuum evaporation. The samples were analyzed by SEM (Inspect S50, Czech Republic) and were observed under 5000x magnification.

RESULTS & DISCUSSION
Temperature and thickness measurement
The data obtained from temperature and thickness measurement from all the tested groups were statistically analyzed by SPSS Statistics 23 (IBM, NY, USA). For temperature assessment, descriptive statistics were done to find the means, standard deviations, maximum and minimum value (Table 1). The pulp chamber temperature rise ranged from 0.4°C to 3°C for the diode laser (940nm) groups. The maximum recorded value was 3°C with 2W group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
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<tr>
<td>0.8</td>
<td>0.7200</td>
<td>0.25298</td>
<td>0.40</td>
<td>1.10</td>
</tr>
<tr>
<td>1.6</td>
<td>1.6800</td>
<td>0.93429</td>
<td>0.60</td>
<td>2.90</td>
</tr>
<tr>
<td>2</td>
<td>1.8090</td>
<td>0.81713</td>
<td>0.80</td>
<td>3.00</td>
</tr>
<tr>
<td>3</td>
<td>1.5100</td>
<td>0.54661</td>
<td>0.50</td>
<td>2.40</td>
</tr>
</tbody>
</table>

To investigate normal distribution of data, Shapiro-Wilk test revealed that data had normal distribution (P>0.05). To examine if the groups were statistically different, One-way ANOVA test was used and the obtained descriptive level revealed that the groups were not significantly different (P>0.05).To find a correlation between temperature elevation and thickness at each group, Pearson test was done. It showed high negative correlation (P<0.01), which revealed that pulp temperature elevation inversely proportional to remnant dentin thickness.

SEM Evaluation
SEM analysis showed that control group presented open tubules and absence of the smear layer (see figure (1)).

For thickness measurement, descriptive statistics were done (Table 2).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean</th>
<th>±SD</th>
<th>SE</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
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<td>0.8</td>
<td>1.873</td>
<td>.134</td>
<td>.042</td>
<td>1.68</td>
<td>2.08</td>
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<tr>
<td>1.6</td>
<td>1.867</td>
<td>.213</td>
<td>.067</td>
<td>1.54</td>
<td>2.20</td>
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<tr>
<td>2</td>
<td>1.830</td>
<td>.151</td>
<td>.048</td>
<td>1.60</td>
<td>2.10</td>
</tr>
<tr>
<td>3</td>
<td>1.897</td>
<td>.154</td>
<td>.049</td>
<td>1.66</td>
<td>2.12</td>
</tr>
</tbody>
</table>

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SEM Evaluation
SEM analysis showed that control group presented open tubules and absence of the smear layer (see figure (1)).

For laser groups, no significant difference was observed from the control group on DTs diameter (figure 2 (a)). Narrowing with some of occluded tubule were observed at 1.6W groups, (figure 2 (b)).While for 2W group, a large

FIGURE 1: Scanning electron microscopic (SEM) views of (control group) treated only with EDTA (17%). The dentin is not covered by the smear layer, the tubules were open. Magnification: 5000x.
number DTs were sealed without signs of cracking or char. (figure 2(c)). At 3W group, laser irradiation provoked excessive melting with some darkened areas indicating dentin carbonization and destruction (figure 2 (d)).

FIGURE 2: Scanning electron microscopic (SEM) views of treated dentin by the diode laser. At 0.8W group no significant difference was observed from the control group on DTs diameter (a). Narrowing with some of occluded tubule were observed at 1.6W group (b). While for 2W group, a large number DTs were sealed without signs of cracking or char (c). At 3W group, laser irradiation provoked excessive melting with some darkened areas indicating dentin carbonization and destruction (d). Magnification: 5000x

DISCUSSION
DH is correlated to the number of exposed tubules on the root surface. The main aim of a successful treatment is the partial or complete occlusion of DTs (Tunar, Gürsoy et al., 2014). In this study we tried to assess the ability of diode laser 940nm laser to reduce or close patent DTs, concerning effects on chamber temperature rise and dentin surface morphology.

A laser wavelength of between 800 and 980nm has a poor absorption in water and hydroxyapatite (Gutknecht, 2007). This low absorption prevails scattering, or diffused transmission of the laser radiation through the dentin reaching the pulp, and important thermal effects (Schoop, Kluger et al., 2006). The low absorbed energy by the dentin surface (via its mineral such as phosphate and carbonate) leads to heat accumulation, which gradually increases the surface temperature (Ying, Gao et al., 2013). This results in denaturation and modification of organic matrix layer with an amorphous form and hence DTs sealing (Marchesan, Brugnera-Junior et al., 2008). We utilized continuous mode to ensure scanning the whole dentin surface, noncontact mode to protect the optical fiber from the resulted heat. SEM analysis revealed that laser effect on the dentin surface was intensified with high power densities, due to increased absorbed energy (Ying, Gao et al., 2013). This reflect increased number of sealed DTs. Approximately optimum sealing of DTs occurred in 2W laser group. But at 3W groups carbonization was noticed indicating irreversible destruction of dentin surface. We measured pulp chamber temperature elevation to confirm that our parameters are within a 5.5°C pulp safety threshold as was established by Zach and Cohen (Zach and Cohen, 1965). The maximum recorded value was 3°C with an average dentin thickness of 1.897 mm, which was accepted with Krnoked, Miletic et al., 2009 study, that 3°C is the maximum threshold to not produce irreversible pulpal damage. At high power 3W a drop in pulp temperature was noticed, which could be due to carbonization that act as heat sink. According to our findings, we suggested that diode laser 940nm irradiation at (1.6, 2) W can be effectively and safely used in the treatment of DTs.

CONCLUSION
Our results confirmed that 940nm diode laser irradiation (1.6, 2) W, continuous mode, irradiation speed: about 1.2 mm/sec for 10 sec, laser fiber tip diameter: 300 Mm can lead to successfully and safely narrowing or sealing DTs. Inversely at high power it could cause surface carbonization and destruction.

RECOMMENDATIONS
Further clinical studies need to be accessed in order to confirm this in vitro result, and to test its long-term treatment effect before final conclusions can be drawn.

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REFERENCES


Metric system
1- mm: millimeter
2- µ: micrometer
3- nm: nanometer
4- mm²: square millimeter
5- cm²: square centimeter
6- W: watt
7- W/cm²: watt per centimeter
8- sec: second
9- min: minute

Symbols
1- %: percent
2- °: degree