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Evaluation and comparison between the bactericidal effect of diode laser irradiation (970 nm) and silver nanoparticles on *Enterococcus faecalis* bacterial strain (an in vitro study)

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Abstract

Background/Aims: Today, the using of diode lasers in dentistry has made a significant progress; it increased the speed of treatment, decreased the time of healing, and showed a bactericidal effect. The thermal effects should be considered in root canal treatment by laser, as the temperature rises to critical levels, causing tissues damage and any thermal change occurs after laser irradiation. The temperature can rise up to 10 °C above the body temperature for less than 2–3 min without damaging the periodontal tissue or burring the tooth structure by using cooling. Antibacterial properties of silver nanoparticles (Ag-NPs) were reducing Gram-positive and Gram-negative bacterial growth, due to a larger surface to volume ratio of nanoparticles. The goal of this study is to evaluate the bactericidal effect of diode laser irradiation (970 nm), the silver nanoparticles in root canals infected by *Enterococcus faecalis* bacteria, and the thermal change that occurs after laser application.

Materials and methods: Forty-five extracted single-rooted human maxillary anterior teeth were collected and used as a container for the test. The samples are inoculated by *Enterococcus faecalis* bacterial strain and randomly divided into three groups: group I (control) ($n = 15$), group II (*Enterococcus faecalis* bacteria and silver nanoparticles) ($n = 15$), and group III (*Enterococcus faecalis* bacteria and diode laser) ($n = 15$). The laser group was divided into subgroups according to the time of laser irradiation (20 s, 30 s, and 40 s).

Results: There was a significant difference between the treated groups, in which the laser group showed a high bactericidal effect than the other groups at the time of radiation 40 s, without damaging the tooth structure or periodontal ligament.

Conclusion: Diode laser with proper parameters is used as an adjunctive endodontic disinfection modality due to its antibacterial effect with a temperature tolerated by periodontal tissues with safety limit.

Keywords: Diode laser-metal nanoparticles, *Enterococcus faecalis* bacteria

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Introduction

The root canal treatment purpose is to eliminate the bacteria and its by-products and prevents the re-entry of the microorganisms into the root canal system after cleaning the root canal (Estrela et al., 2014). Using mechanical and traditional methods is not enough for cleaning the root canal and dentinal tubular system (Byström & Sundqvist, 1981; Dalton et al., 1998), and excellent cleaning of the dentinal tubules becomes more difficult (Nair et al., 1990); so recently, the dentist used diode laser in the clinic for different dental applications (Mortiz et al., 1998; Pendyala et al., 2017; Yeh et al., 2005).

The diode laser is a semiconductor laser, and its wavelength for dental use ranges between 800 and 1000 nm that emits in a continuous and pulsed mode using an optical fiber as the delivery system (Camargo, 2011). Bacterial reduction and less apical leakage become important to this system and for endodontic treatment. The principal laser action is photo-thermal. The high-power diode laser is used to kill bacteria (El-Batanouny et al., 2008) and stop bacterial growth by thermal effect and closure of the irradiated dentinal tubules (Ashofteh et al., 2014; Kaiwar et al., 2013a).

The laser releases a large amount of energy that is transformed into heat, which is causing damage to the tooth structure, while overheating lead to irreversible changes as melting, microfractures, and carbonization (Alfredo et al., 2008; Gutnecht et al., 2005). The temperature on the outer root surface and apex within 7–8 °C does not cause damage, but if the temperature rises more than 10 °C without cooling, it causes damage in the surrounding periodontal and bony structures (Ribeiro et al., 2007; Hussey et al., 1997).

Nanotechnology has been used in dentistry to provide high mechanical properties and bactericidal effects. These materials as silver nanoparticles have antimicrobial properties and compatible physical properties when compared with conventional materials due to their small size and increased surface area (Rodrigues et al., 2018a; Anil et al., 2017).

Silver nanoparticles (Ag-NP) have a high bactericidal effect, which is widely used in health sciences (Alabdulmohsen & Saad, 2017). Recently, it is used to prevent bacterial growth in different fields and applications, as in dentistry (García-Contreras et al., 2011; Rodrigues et al., 2018b); its antibacterial effect is due to the reduction in particles' size (Krishnan et al., 2015; González-Luna et al., 2016) and they have self-cleaning properties (Rodríguez-Chang et al., 2016), by effect on Gram (+) and Gram (–) bacteria, producing damage to the microorganism's membrane (Daming et al., 2014).

Enterococcus faecalis is the most common bacteria leading to failures of root canal treatment (Monawer & Abdulkahar, 2016; Colaco, 2018; Haypek, 2006) resisting

the antibacterial agents such as NaOCl in different treatments (Valera et al., 2009; Sohrabi et al., 2016). *E. faecalis* can form a biofilm and enter inside the dentinal tubules (Duggan & Sedgley, 2007; Rahul et al., 2014), resistance to alkaline pH and to calcium hydroxide pastes, which normally inhibit other bacteria. This resistance may be related to severe and excessive working active proton pump in the membrane of these bacteria (Weckwerth et al., 2013).

This study showed the bactericidal effect by using diode laser (970 nm), and silver nanoparticles on *Enterococcus faecalis* bacterial strains and changes occur in the tooth structure after laser irradiation.

Material and methods

Sample selection

A total of 45 extracted single-rooted human maxillary anterior teeth were collected and scaled to remove all adhering soft tissues and kept in saline solution (0.09%) before the experiment.

Tooth preparation

The teeth decapitated at the level of cemento-enamel junction (CEJ) using a high-speed diamond disk to facilitate the mechanical preparation of the root canals to be used as a container to perform the test. The working length was established by subtracting 1 mm from the apical foramen. The canals shaped using Gates Glidden drills (Main Inc., Japan) # 4 and # 5, which were used to flare out the coronal part of the canal by maintaining 1 mm of the apical constriction for enough width that will able to contain 25 ml of bacteria and 25 ml of metallic nanoparticles (Ag-NPs). For complete removing of the smear layer, each root canal was irrigated using 17 ml of 17% EDTA (for 2 min) (Oakland Street, Watertown, MA 02272-0780, USA). The teeth were then allowed to air-dry overnight at room temperature, and the apical foramen was sealed externally and waterproofed with two coats of clear nail polish (Kaiwar et al., 2013b). The teeth were placed in the Eppendorf to put them into an autoclave (Melatron 23, Melag, Berlin, Germany) for 20 min at 121 °C under 1.2 psi pressure for sterility by removing all pre-existing bacteria (Gutkecht et al., 2000; Radaelli et al., 2003). All samples inoculated with *Enterococcus faecalis* bacterial suspension. The samples were randomly divided into three groups: group I (control) ($n = 15$), group II (*E. faecalis* bacteria + Ag-NPs) ($n = 15$), group III (*E. faecalis* bacteria + diode laser irradiation) ($n = 15$). Each group was divided into three subgroups according to the time of diode laser irradiation (970 nm) (20 s, 30 s, and 40 s) ($n = 5$) at 1 W in continuous mode. The teeth covered by wet sterile cotton of saline to keep the environment during procedure moisture and then placed in a sterile Eppendorf to be ready for the next procedure.

Preparation of media

One liter of culture agar media prepared by using 90 g of BHI agar (Acumedia, Baltimore, Maryland, USA) in 1000 ml distilled water, then heated to the boiling point to dissolve the medium completely. It dispensed and sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 min, and then, it cooled to 50–55 °C and 1 ml of sterile and 1% of potassium telluride solution were added to the medium. It was mixed well and poured into sterile Petri dishes. The solution was allowed to cool down and then maintained in the refrigerator until use to avoid any bacterial contamination.

Bacterial suspension preparation and bacterial inoculation

The bacterial suspension prepared from reference strain of *Enterococcus faecalis* (ATCC 29212) bacteria, which prepared in BHI agar and then adjusted to 0.5 McFarland units [1.5×10^8 (CFU/mL)]. Twenty-five milliliters of bacterial suspension in BHI broth applied to all samples of root canals using a sterile micropipette with a sterile needle in group I. Twenty-five milliliters of Ag-NPs was added in the root canals of group II. Group III was subjected to laser irradiation treatment at three different times (20 s, 30 s, and 40 s), and the bacterial count was detected after laser irradiation (Hong-gang et al., 2002).

Bacterial count

Enumeration of bacteria was carried out after making serial dilution up till 10⁷ of the inoculums, and from each diluted inoculation, 1 ml of the culture was plated on the surface of BHI agar plates and then incubated aerobically at 37 °C for 24 h. After incubation, enumeration of bacteria was carried out from countable plates (30–300 CFU/ml) (Sieuwert et al., 2008).

Laser treatment

Group III was irradiated using a diode laser (Siro Laser Advance class III b, SIRONA Dental Company, Germany) at wavelength 970 nm and output power at 1 W in a continuous mode. It provided through a flexible 220-ml fiber optic diameter with a special handpiece. The fiber optic was disinfected for each use by 70% ethyl alcohol and inserted inside the cavity with a spiral continuous movement clockwise from the top to the floor and anti-clockwise in a reverse direction (Al-habeeb et al., 2013). This procedure equally distributes the laser energy inside the cavity to avoid excessive heat generation and carbonization in the internal cavity surface. Irradiation time was (20 s, 30 s, and 40 s). A scanning electron microscope (SEM) was used to examine the changes of the tooth structure in each group after laser application (Fig. 1).

Bacteriological evaluation of the treated samples

Colony-forming units (CFUS) were used to detect bacterial count after silver nanoparticle addition as well as laser irradiation. After laser irradiation, 50 ml of suspension solution was placed on a Petri dish and then incubated aerobically in the incubator for 24 h at 37 °C. The grown colonies in all groups were identified and counted (Sieuwert et al., 2008; Scott, 2011). Upon irradiation, the specimens were placed into sterile Eppendorf tubes and 100 ml of physiological saline solution was added. The extracted fluid was diluted in log 10 steps, and then, 25 ml of each dilution was applied to the culture plates and then incubated at 37 °C for 24 h. The colonies were counted and the total number of bacteria (CFUS/ml) was assessed (Colony Star, Funke Gerber Product, Gebr Liebsch, Germany) (Lee et al., 2006; Johnsrud, 2002).

Results

Statistical analysis

The data was presented as mean and standard deviation (SD) values. One-way ANOVA test was used to compare the different treatment modalities as well as the different time periods. Tukey's test was used for pairwise comparisons between the mean values when ANOVA test is significant. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM¹ SPSS² Statistics Version 20 for Windows (Table 1).

The effects of different treatments and different times in each treatment on the bacterial counts are as follows:

A. For the effect of different treatment, there were statistically significant differences between the groups. The laser group showed the statistically significant lowest mean bacterial counts, followed by the silver group. The control group showed the highest mean of bacterial count, and there was no statistically significant difference between times.

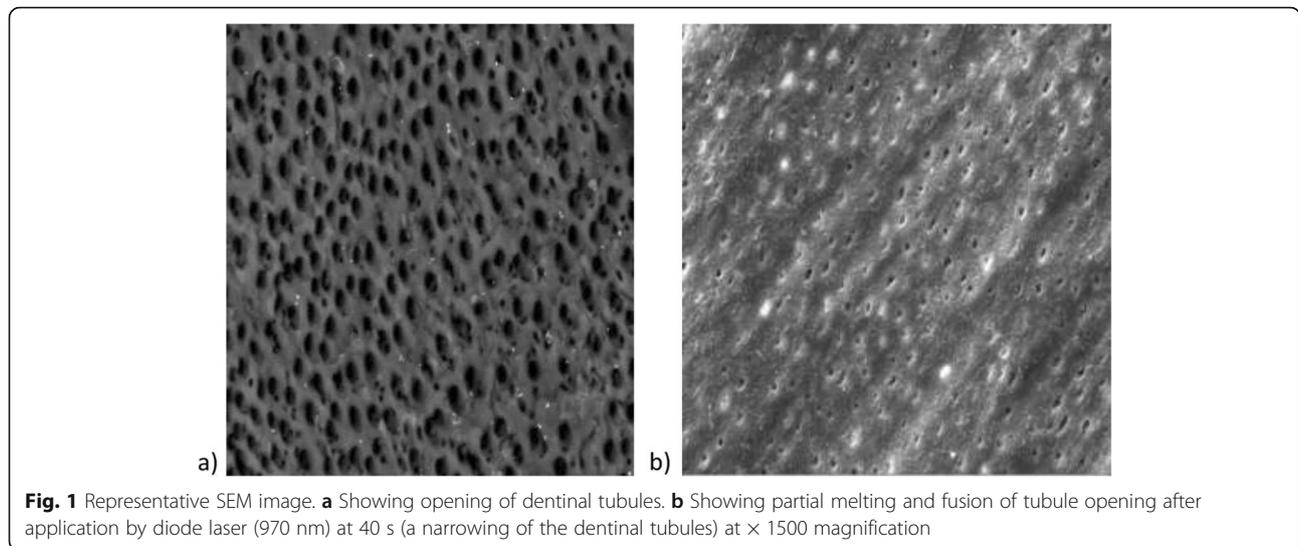
B. For the effect of different times within each treatment on the bacterial counts, the laser group showed the statistically significant lowest mean of bacterial counts, and there was also no statistically significant difference between 20 s and 30 s; both showed statistically significant higher mean counts, while 40 s showed the statistically significant lowest mean bacterial count.

In silver nanoparticle group, there was no statistically significant difference between 20 s and 30 s; all showed a highly statistical significant difference on mean bacterial counts, while the 40-s period showed the statistically significantly lowest bacterial count.

The diode laser group showed higher bactericidal effects than the other groups by increasing at the time of laser irradiation. In SEM examination for tooth structure at 40 s,

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the narrowing in the opening of dentinal tubules occurs without melting or carbonization of the tooth structure.

Discussion

Diode laser available in red and infrared spectrum. Near-infrared (NIR) lasers are characterized by a high absorption of the mineral present in the tooth structure and chromophore present in the soft tissue. In this study, we selected 970 nm wavelengths, which are most commonly used wavelengths in dentistry. However, the body can withstand temperature up to 10 °C of its temperature (37 °C); according to Gutkecht et al. (2000), the tooth structure can tolerate temperature without causing any damage to the root or the adjacent structures, as the effect of diode laser treatment on the tooth structure depends on pulse energy, frequency, spot size, and wavelength. Baburao et al. (2014) in agreement with our, they used continuous mode of radiation, because it is more easy for the dentist to irradiate the whole dentin surface by this way, in which the heat conduction between the periapical tissues and the root canal depended on the thickness of

the dentinal walls, while the passage of heat becomes more difficult in the thick wall of the root canal. In our study, the mechanism of the diode laser (970 nm) on dentin, in agreement with Umana et al. (2013), in which a part of the energy absorbed by dentin minerals such as phosphate and carbonate, causing thermo-chemical ablation and occlusion of dentinal tubules. Measuring the rise in temperature during laser irradiation to study the thermal effect on the tooth structure and the periodontal tissues, according to the studies of Anic et al. (1996), in our study, laser irradiation heat increased within the body temperature tolerance without causing any damage to the tooth structure, just a narrowing of the dentinal tubules was observed at delivered output powers of 1 W at 40 s for 970 nm diode laser, which is in agreement with Rosenberg et al. (1971), who reported that the temperature measurements at 810 and 980 nm diode laser irradiation up to 2 W cannot be dangerous to the tooth surface and pulp tissues. Alfredo et al. (2008) demonstrated that power out outputs ranging from 0.5 to 2.5 W and irradiation times from 10 to 120 s, the temperature rise in continues mode than the body temperature which is safe for the tooth structure and the surrounding structure which is in agreement with our result that the temperature rise might depend not only on the irradiation mode but also on the tooth anatomy at 40 s because there is no contact between the fiber and dentinal walls. Our results in agreement with Ribeiro et al.’s (2007), who used diode laser with the same parameter and correlated to the lower microleakage due to occlusion of dentinal tubules obtained by Gutnecht et al. (2005).

This study was conducted to evaluate the antibacterial effect of diode laser and silver nanoparticles which have the ability to kill bacteria at different times of exposure for disinfection of contaminated

Table 1 The mean, standard deviation (SD) values, and one-way ANOVA test for comparison between bacterial counts with different treatments at different times

Time (s)	Group						P values
	I		II		III		
	Mean	SD	Mean	SD	Mean	SD	
40	220.2 ^a	20.7	15.01 ^a	0.60	8.1 ^b	2.1	< 0.001*
30	206.3 ^a	25.9	22.00 ^a	0.54	27.6 ^b	3.9	< 0.001*
20	155.2 ^a	27.2	28.04 ^a	0.50	35.5 ^b	4.5	< 0.001*
P value	0.987		0.194		0.060		

Different letters in the same row are statistically significantly different
 *Significant at P ≤ 0.05

samples as per Gerek et al. (2010). Therefore, our results in laser group III demonstrated that the bacterial counts decreased by increasing the time of laser exposure within few seconds which is in agreement with Ramskold et al. (1997). The findings agreed with this study that, as the time of 40 s of laser exposure, the diode laser could remove the *E. faecalis* without inducing a high-temperature rise as in Lee et al. (2006). The mechanisms regarding the antibacterial effect of diode laser are that the thermal effect was considered the main reasons for the laser to kill the bacteria (Radaelli et al., 2003). In our study, the cell growth inhibited after exposure to laser irradiation by destruction of the cell wall prevents the cell growth and cell lysis, which is in agreement with Barbosa et al. (2008); also, the thermal effect of the laser causes stress on the cells to prevent the accumulation of protein debris causing cell death (Pimpang et al., 2008; Ahmed et al., 2011).

Ag-NPs have an antibacterial effect against both Gram-positive and Gram-negative bacteria as in Rai et al. and do not allow developing resistance (Rai et al., 2012). In silver nanoparticle group II, it showed antibacterial effect which increases by time. It is in agreement with Morones et al. (2005) in which positively charged Ag-NPs interact with the negatively charged bacterial cell walls and adhere and penetrate into the bacterial cell leading to the loss of cell wall and permeability. It showed in our study that Ag-NPs could induce cytotoxicity and inflammatory response in cells resulting from damage to enzymatic systems and DNA leads to cell death in agreement with Guzman et al. (2012). The results of this study showed that both of the diode laser and silver nanoparticles showed an antibacterial effect, but diode laser showed more effective to remove more of bacterial counts than silver nanoparticles.

Conclusion

The results of the study showed that the diode laser 970 nm has a strong antibacterial with the investigated parameters in comparison to silver nanoparticles; however, the effectiveness of silver nanoparticles in bacterial reduction was acceptable as a less costly method.

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Authors' contributions

Both authors participated in the development and implementation of the research plan and subsequently wrote it. Both authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors state that they all want to publish this paper in the bulletin of the National Research Centre.

Competing interests

The authors declare that they have no competing interests.

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