



# Efficacy of diode laser and sonic agitation of Chlorhexidine and Silver-nanoparticles in infected root canals

Eficácia do laser de diodo e agitação sônica de clorexidina e nanopartículas de prata em canais radiculares infectados

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## ABSTRACT

**Objective:** To assess the efficacy of agitation of chlorhexidine (CHX) and Silver nanoparticles "AgNps" with 810nm diode laser or sonic endoactivator compared to side-vented needle on infected root canals with *Enterococcus "E" Faecalis* biofilms. **Material and Methods:** Sixty-five extracted human premolars with single oval canals were instrumented by protaper system up to F3. Biofilms of *E. faecalis* were generated based on a previously established protocol. Two teeth were used to check the biofilm formation, then the remaining Teeth were randomly divided into three equal experimental groups according to agitation techniques used: group 1 (810 nm diode laser with 1 watt), group 2 (sonic endoactivator) and group 3 (Side vented needle). Each group was further divided into three equal subgroups according to the irrigant solution into; subgroup A: chlorhexidine, subgroup B: silver nanoparticles and subgroup C: distilled water. Confocal laser scanning microscopy "CLSM" was used to assess bacterial viability. Data were analyzed by appropriate statistical analyses with  $P = 0.05$ . **Results:** Regarding the activation method, all groups had a significantly high percentage of dead bacteria ( $P < 0.05$ ). However, Laser was significantly the highest and Endoactivator the least ( $P < = 0.001$ ). Diode laser agitation of AgNps irrigant showed the highest reduction percentage of bacteria (78.1%) with a significant difference with both CHX and water irrigation. **Conclusion:** Under the condition of the present study; results reinforced that laser activation is a useful adjunct, 810 nm diode laser agitation of AgNps or chlorhexidine was more effective in disinfection of oval root canals than endoactivator and side vented needle techniques.

## KEYWORDS

Dentinal infection; Silver-nanoparticles; chlorhexidine; agitation; Diode Laser; Sonic endoactivator.

## RESUMO

**Objetivo:** Avaliar a eficácia da agitação de clorexidina (CHX) e nanopartículas de prata (AgNps), com laser de diodo de 810 nm ou endoativador sônico, em comparação à agulha de ventilação lateral, em canais radiculares infectados com biofilmes de *Enterococcus "E"; Faecalis*. **Material e Métodos:** Sessenta e cinco pré-molares humanos com um único canal oval, extraídos, foram instrumentados pelo sistema protaper até F3. Os biofilmes de *E. faecalis* foram gerados com base em um protocolo previamente estabelecido. Foram utilizados dois dentes para verificar a formação do biofilme, e os dentes restantes foram divididos aleatoriamente em três grupos experimentais iguais, de acordo com as técnicas de agitação utilizadas: grupo 1 (laser de diodo 810 nm com 1 watt), grupo 2 (endoativador sônico) e grupo 3 (Agulha com ventilação lateral). Cada grupo foi dividido em três subgrupos iguais, de acordo com a solução irrigante; subgrupo A: clorexidina, subgrupo B: nanopartículas de prata e subgrupo C: água destilada. A microscopia confocal de varredura a laser foi usada para avaliar a viabilidade bacteriana. Os dados foram analisados por análises estatísticas apropriadas com  $P = 0,05$ . **Resultados:** Em relação ao método de ativação, todos os grupos apresentaram percentual significativamente alto de bactérias mortas ( $P < 0.05$ ). No entanto, para o laser foi significativamente o mais alto e, para o endoativador, o menos alto ( $P < = 0.001$ ). A agitação com laser de diodo irrigante AgNps apresentou a maior porcentagem de redução de bactérias (78,1%), com diferença significativa tanto para irrigação com CHX quanto com água. **Conclusão:** Sob as condições do presente estudo; os resultados reforçaram que a ativação a laser é um complemento útil, a agitação por laser de diodo de 810 nm de AgNps ou clorexidina foi mais eficaz na desinfecção dos canais radiculares ovais do que as técnicas de endoativador e agulha com ventilação lateral.

## PALAVRAS-CHAVE

Infecção dentinária; Nanopartículas de prata; clorexidina, bagitação; Laser de Diodo; endoativador sônico.

## INTRODUCTION

One of the main challenges facing the dentists in the endodontic steps is how to totally disinfect the root canal [1]. *E. faecalis* is the most prominent microorganism involved in persistent infections after root canal therapy. *E. faecalis* has the ability to penetrate the dentinal tubules and cementum. It can survive in biofilm form at anatomical complexities of root canal system, over the foreign bodies like gutta-percha or other obturating materials extending into periapical tissues and can survive for prolonged periods under nutrient-depleted conditions [2].

Trying to overcome the challenge compulsory by the presence of biofilm and reach complete disinfection or significant bacterial reduction in the root canals, many irrigants have been indicated during endodontic treatment. Chlorhexidine gluconate (CHX) at 2% is one of the most commonly used irrigants and considered as an effective antimicrobial agent. It has many properties; broad-spectrum, substantively (extended outstanding activity) and a relative absence of toxicity that recommend it to be used as an endodontic irrigant [3, 4].

Silver nanoparticles (AgNps) have gained popularity because of their unique ability to penetrate tissues, interact with bacteria, exhibit potent antimicrobial activity and biocompatibility [5].

The agitation of irrigating solutions with the laser has become common [6]. Researchers examined many laser systems to attain complete disinfection of root canal system and the adjacent dentinal tubules and yet there is still argument about which is the most powerful laser system in regards to providing a sterilized root canal system. The high bactericidal effect of diode laser has been reported in multiple studies [7]. Therefore, due to the bactericidal effect of diode laser

and its low cost compared to other commonly used laser systems in endodontic, it can be used in agitation of the irrigation during the mechanical debridement procedures [8].

The Endoactivator System "EA" is a sonically driven irrigant activation system planned to produce dynamic fluid agitation inside the canal that has been shown to improve the effectiveness of irrigation better than the traditional syringe irrigation [9]. So the present study highlighted the effect of 810 nm diode laser and sonic agitation on two types of irrigants; [chlorhexidine (CHX) and silver nanoparticles (AgNps)] applied in root canals.

## MATERIAL AND METHODS

This research was approved by the Ethics Committee of National Institute of Laser Enhanced Sciences (NILES), Cairo University, Egypt (CU/NILES/30/19). Also this study was done in the microbiology and molecular biology department, faculty of medicine, Cairo University.

### Selection and Mechanical preparation of samples

Sixty -five extracted human premolars with single oval canal and fully formed apices were selected from hundred extracted teeth for periodontal reasons. Each root was digitally radiographed using RVG 6200 digital sensor (Carestream, Rochester, New York, United States) and measured by its software to select canals with ovality ratio more than 2.5. The crowns were sectioned such that roots were standardized to 12 + 2 mm and stored in labeled vials at 100% humidity.

Preparation was performed using the ProTaper system (Dentsply-Maillefer, Ballaigues, Switzerland), following the sequence S1, S2, F1, F2, and F3). Using X – smart motor at 300 rpm/ 2. Ncm. (Dentsply,

Switzerland) [10]. At every instrument change, the canals were irrigated with 5 ml 2.5% sodium hypochlorite "NaOCl" solution (Clorox, Nobelwax Factories for chemicals, Egypt) using 5 ml –syringe with 30 –G side-vented needle (Canal Clean, Biodent, Co, Ltd, Pagu City, Korea) which inserted 2 mm short of working length.

#### **Final irrigation and sterilization of the samples**

Final irrigation was performed with 5 ml 15% Ethylenediaminetetraacetic acid "EDTA" (Endo-Solution, Cerkamed, Stalowa Wola, Polska) for 3 min and 5 ml NaOCl for 3 min to remove the smear layer, and then the canal was irrigated with 10 ml of physiological saline solution to remove the EDTA. Finally, the teeth were sterilized in the autoclave at 121°C for 30 min .

#### **Inoculation and incubation of the teeth with *E faecalis***

Using a sterile micropipette, twenty microliters of *E. faecalis* suspension (matching McFarland's turbidity of tube no 0.5) was syringed into each root canal. Each inoculated root was kept in separate sterile test tube with caps in a rack. Then incubated at 37°C for 7 days, to allow the proliferation of microorganisms, and their further penetration into the dentinal tubules and formation of biofilms.

#### **Classification of the samples:**

Two teeth were used to check the biofilm formation, then the remaining teeth (63) were randomly divided into three equal experimental groups according to agitation techniques used: group 1 (810 nm diode laser with 1 watt), group 2 (sonic endoactivator) and group 3 (Side vented needle). Each group was further equally divided into three subgroups according to the irrigant solution into; subgroup A: chlorhexidine, subgroup B:

silver nanoparticles and subgroup C: distilled water:

The agitation mechanisms in the three groups were as a follow:

#### **Group 1 (diode Laser)**

Agitation was done with 810 nm Diode laser with continuous mode and output power 1 watt (Zolar lasers, Canada) which delivered into 200 um flexible plain endodontic fiber. The fiber was inserted parallel to root canal wall and used with helicoidally movement in apical-coronal directions. Agitation was done for 10 seconds to 1 ml irrigant. The sequence was repeated 5 times, giving 5 ml total volume and 50 sec total agitations.

#### **Group 2 (Sonic endoactivator)**

In this group agitation was done by Sonic endoactivator device (Dentsply, Tulsa) with red polymer tip # 25/0.04 at speed 10000 rpm. The samples in this group were irrigated with 5 ml of an irrigating solution, where each 1ml of the irrigant was followed by 10 seconds of sonic agitation [11].

#### **Group 3 (side –vented needle)**

Side vented needle delivered 5 ml irrigant during moving slowly up and down along canal length. All agitations were performed at 2 mm away from the working length.

#### **Confocal laser scanning microscopy (CLSM) examination**

Sixty –five roots were sectioned for detecting viable and nonviable bacteria on root canal walls. Roots were set in blocks of fast setting acrylic resin and two lines were drawn with a marker on the exposed part of the root, one buccal and one palatal for vertically sectioning into two halves, approximately parallel to the tooth axis, utilizing the microtome saw. A sample from each sectioned root was taken for scanning. Each sample was stained by both Acridine

orange (AO) and Propidium iodide (PI) dyes separately, just before the CLSM examination. Zeiss LSM 710 confocal microscope (Carl Zeiss, Germany) was used with 40 x objectives for scanning. Three random areas of the middle third of the root canal were scanned with a 2-mm step size by the CLSM. For each image the median intensity of green and red bacteria were calculated by the soft-ware (green for live and red for dead bacteria), this number was tabulated and statistically analyzed.

#### Data presentation and analysis

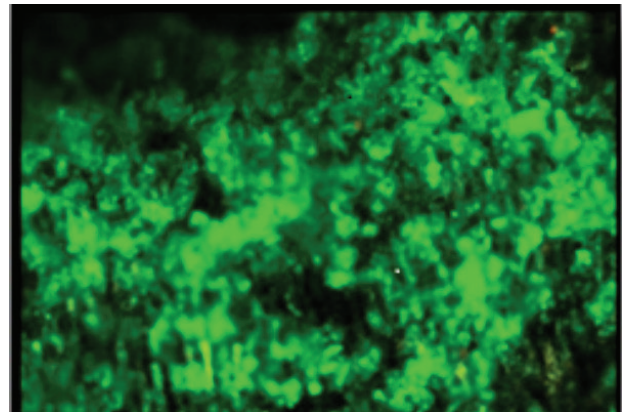
To identify the effect of activation protocols on bacterial reduction (dead cell %) in each irrigation protocol, one-way ANOVA was applied. Bonferroni's correction for multiple testing was used in one-way analysis of variance.  $P = 0.05$  for the analyses. Two-way ANOVA was performed to weigh the effect of the irrigation protocol and activation method as the two independent variables on the outcome (percentage of dead cells. Data was analyzed using IBM SPSS advanced statistics (Statistical Package for Social Sciences), version 21 (SPSS Inc., Chicago, IL).

## RESULTS

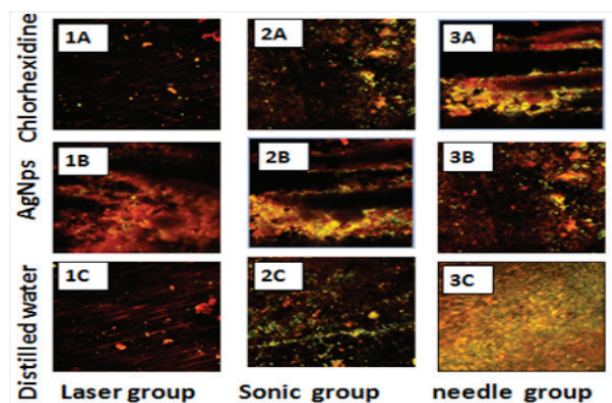
The data obtained from the CLSM are tabulated (Table I). Figure 1 shows a homogenous penetration of *E. faecalis* deep into the dentinal tubules of the root canal. figure 2 shows biofilm destruction within the root canal lumen for all groups. Regarding the activation method, all groups had a significantly high percentage of dead bacteria ( $P < 0.05$ ). However, Laser was significantly the highest and Endoactivator the least ( $P \leq 0.001$ ).

Regarding the irrigant type; AgNps activated with diode laser had the highest percentage of dead bacteria (78.1%) followed by needle agitation (76.47%) then sonic (72.94%). Without significant difference ( $p > 0.05$ )

In chlorhexidine subgroup, the high percentage of dead bacteria was in laser group (71.81%) followed by needle group (70.18%) then sonic group (68.99) Without significant difference ( $p > 0.05$ ). while distilled water subgroup showed high percentage of bacterial reduction in laser group (71.46%) followed by sonic (62.7847%) then needle group (60.6%) with significant difference between laser and sonic and laser with needle ( $p < 0.05$ ) while there was no significant difference between sonic and needle ( $p > 0.05$ ).



**Figure 1** - Representative Confocal laser scanning microscope (CLSM) image of *E. faecalis* within dentinal tubules



**Figure 2** - Representative confocal laser scanning microscope (CLSM) images in Laser, sonic and needle activation groups with chlorhexidine or AgNps or distilled water irrigant showing the distribution of live (green fluorescence) and dead bacteria (red fluorescence) within the dentinal tubules. (1A) CHX activated by laser, (2A) CHX activated by sonic, (3A) CHX activated by needle, (1B) AgNps activated by Laser, (2B) AgNps activated by sonic (3B)) AgNps activated by needle, (1C) Distilled water activated by laser, (2C) Distilled water activated by sonic and (3C) Distilled water activated by needle.

**Table 1** - percent of apparently dead bacterial cells in the biofilm within the dentinal tubules, assessed by confocal laser microscopy after three different activation techniques with three different irrigant.

Group	Subgroup	Mean %	Std.Deviation	P value
Laser*#	CHX	71.8143 <sup>a</sup>	4.03276	0.018
	AgNps	78.0514 <sup>c</sup>	3.94723	
	H2O	71.4565	4.82094	
Sonic	CHX	68.9947 <sup>c</sup>	2.33525	0.002
	AgNps	72.9405 <sup>b</sup>	3.58203	
	H2O	62.7847 <sup>d</sup>	7.55672	
Needle	CHX	70.1782 <sup>ab</sup>	3.73252	0.044
	AgNps	76.4703 <sup>f</sup>	6.55219	
	H2O	60.6	3.2501	

(\*) means significant between laser and sonic in H2O irrigant  
 (#) means significant between laser and needle in H2O irrigant  
 (a) Means significant CHX and AgNps within the same group  
 (b) Means significant between CHX and H2O within the same group  
 (c) Means significant between AgNps and H2O within the same group.

## DISCUSSION

The main goal of endodontic treatment is the complete disinfection of the root canal system; however, it is difficult due to complex anatomy of the root canal system and biofilm mediated infection. *E. faecalis* was chosen because it is generally believed that it is one of the most resistant microorganisms found in the infected root canals and endodontic treatment failures [9, 10].

The clinical challenge to deliver irrigants into unprepared infected canal extensions; as well as the most apical infected segment recommended the use of automated agitation of irrigant in disinfection of root canal systems [7]. So the present study evaluated the antibacterial effect of activated agitation of diode laser, sonic and side-vented needle with three types of irrigants; Chlorhexidine, AgNps, and distilled water in infected oval root canal with *E. faecalis*. Chlorhexidine (CHX) was selected in the present study as it has a broad-spectrum antimicrobial effect and kills *E. faecalis* in the dentinal tubules. [9]. .as well as AgNps exhibit potential antibacterial activity

and does not allow to develop resistance [11] Positively charged AgNps interact with the negatively charged bacterial cell walls, adhere, and penetrate into the bacterial cell leading to the loss of cell wall integrity and permeability [ 12-17].

Diode laser induced cavitation and side vented needle non laminar streaming probably provided better mechanical turbulence to penetrate infected dentin and effectively carry away the microorganisms than acoustic streaming by vibrating inserts of endoactivator [18-20].

In the present study the lasing protocol favored the disinfection of the root canal. Through limited studies existed specially on 810 nm diode laser; generally, diode agitation seems promising [6, 18]. Agitation of AgNps by 810 nm diode laser I watt for 50 sec improved *E. faecalis* eradication compared to sonic or needle agitation. Using 10 sec continuous 810 nm diode laser which repeated five times for agitation of AgNps improved *E. faecalis* eradication by 78.05% however inadequacy of diode laser to remove more percentage of bacteria may be due to using of low output power (1 watt) which is in accordance with other studies [21-26].

Sonic agitation produced bactericidal effect in the present study probably due to oscillating movement which allow hydrodynamic circulation of the irrigant. A reduced antibacterial efficiency of sonic compared with other techniques may be due to the greater displacement amplitude of the small vigorously vibrating polymer tip, also due

To weakened. Currents, impeding microstreaming and irrigant activation in apical part of the root beyond the vibrating tip [27-29].

In the present study, laser activated agitation was more efficient than endoactivator

[18]. Endoactivator in the present study was less efficient than needle agitation this may be due to vacuum and remove of irrigant before replenishing [30]. Side-vented needle agitation came after laser and better than sonic as vertical up and down movements of the needle allowed distributing the localized high dynamic flow at the side exit along the oval canal allowed effective reflux of irrigant coronally as previously reported [30].

The enhancing effect of 810 nm diode laser agitation in disinfection of oval canals can be complemented by a further study to evaluate other different protocols in canal disinfection with different irrigant and providing better bactericidal effect.

## CONCLUSIONS

Under the condition of the present study; the results reinforced that laser activation is a useful adjunct, 810 nm diode laser agitation of AgNps and chlorhexidine was more effective in disinfection of oval root canals than endoactivator and side vented needle techniques.

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d is suitable for predicting the chronological age, whereas Fishman method is accurate to estimate the skeletal maturity stages, since it represents a clear and easily identified SMI stages [11].

### Study limitation

The regression models suggested in this study need to be validated.

## CONCLUSIONS

Within the limits of this study results, it is concluded that there is a strong correlation between chronological age and skeletal age estimated by Fishman method and dental age estimated by Nolla method. Yemeni children are delayed in skeletal maturity and dental development when compared to Fishman and Nolla age standards. Chronological age of Yemeni children is unsuitable dependent timing for orthodontic intervention. Skeletal age remains essential for clinical orthodontic practices, while dental age estimated by Nolla method is a helpful mean for prediction of unknown chronological age for Yemeni boys and girls aged between 13 and 16 years for civil and forensic purposes.

### Financial or other competing interests:

None.

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