

# ASDJ

AINSHAMS DENTAL  
JOURNAL

Print ISSN 1110-7642

Online ISSN 2735-5039

AIN SHAMS DENTAL JOURNAL

Official Publication of Ain Shams Dental School

March 2021 • Vol. XXIV

## Effect of Two Laser Systems on Root Canal Disinfection: An in Vitro Study

Mohamed M.N. El-Tayeb<sup>1</sup>, Mohamed Nabeel<sup>2</sup>

### Abstract

**Purpose:** Successful endodontic therapy is based mainly upon effective mechanical instrumentation and disinfection of root canals. This study compares the antibacterial effect of Erbium, chromium: yttrium-scandium-gallium-garnet (Er, Cr:YSGG) laser 2780nm wavelength, diode laser 940nm wavelength and sodium hypochlorite (NaOCl) 5.25% solution on *Enterococcus faecalis* (*E. faecalis*) biofilm.

**Materials and methods:** A total of 50 extracted human permanent maxillary central incisor teeth were prepared to size X4 Protaper Next and contaminated with *E. Faecalis* for biofilm formation. After ten days of incubation and based on the method of disinfection of the root canals, the specimens were randomly grouped into; group A (n=15), which was irradiated by ER, Cr: YSGG laser 2780nm wavelength; group B (n=15), which was irradiated with a diode laser 940 nm wavelength; group C (n=15), which was rinsed with 5.25% NaOCl solution. Three teeth were used to confirm the biofilms formation by scanning electron microscope (SEM) and two teeth served as negative control. Intracanal bacteria sampling was performed under complete aseptic conditions before and after disinfection. The specimens were cultured to enumerate the colony forming units (CFUs) count.

**Results:** Although group C (5.25% NaOCl solution) showed a more disinfection than both laser systems, there was no significant difference between all groups ( $P > 0.05$ ).

**Conclusion:** Both ER, Cr: YSGG laser 2780nm wavelength and diode laser 940nm wavelength have nearly similar antibacterial efficacy on *E. faecalis* compared with NaOCl 5.25% solution. The use of laser for root canal disinfection may be advantageous considering several unfavorable actions of NaOCl.

**Key words:** Laser, *Enterococcus faecalis*, Root canal, Sodium hypochlorite.

1. Lecturer, Department of Endodontics, Faculty of Oral and Dental Medicine, Badr University in Cairo, Cairo, Egypt.
2. Lecturer, Endodontic Department, Faculty of Oral and Dental Medicine, Misr International University, Cairo, Egypt.

**Introduction:**

Success of the root canal therapy (RCT) depends mainly upon the efficacy of mechanical instrumentation and disinfection of the root canals (1). Mechanical instrumentation may leave some undebrided areas in the root canals. Presence of microorganisms in these areas plays a critical role in development of pulpopariapical pathoses (2,3).

Persistent periapical pathoses are usually associated with *E. faecalis* (1,4,5). *Enterococcus faecalis* is commonly isolated from persistent endodontic infections. However, isolation of *E. faecalis* periapical primary infection is lower than the persistent infections (2,4). This microorganism is usually isolated from endodontic infections as pure culture or mixed with some species of bacteria and/or fungi but it is usually the dominant species (5,6).

Several chemicals and/or lasers were applied for sterilization of the root canals and eradication as many microorganisms as possible. No single procedure was reported as an efficient technique (7). Although sodium hypochlorite is the gold standard for root canal disinfection, it has many disadvantages such as the lack of substantivity and smear layer removing ability (1). The ability of chemicals to spread into the root canals determines their antibacterial effects (8). Therefore, search for efficient disinfection technique is continuing due to the continuous increase in antibiotic resistant strains and side effects produced by the used chemicals (9,10).

Ruby laser was developed by Maiman in 1960. Then, application of laser in the endodontic therapy was recorded firstly by Weichman in 1971 (11). Several studies on the potential benefits of laser in endodontics have been published. Several types of high-powered laser have a bactericidal action. This power differs between the various lasers. In addition, when using laser in

endodontics, it may reflect, scatter and/or transfer to the neighboring tissues (12). Lasers have characteristic benefits in endodontic therapy and direct contact between the microorganisms and fibre tip has no necessary. Therefore, production of the laser energy represents a method for disinfection of the dentinal tubules. Also, lasers are able to remove the smear layer and tissue debris from the canal wall (12).

With the recent advances in laser fibres, the use of laser in RCT has been increased in last years (12). Laser can be transported into root canal system by fiber optics like diode lasers or with hollow tubes like Er-YAG lasers. The difference in the delivery techniques affects the ability of same lasers in disinfection of the root canal system (11).

Researchers assessed different laser systems to obtain an ideal endodontic disinfection. There is controversy about the effectiveness of the laser in root canals disinfection. Therefore, the current work compares the antibacterial action of ER, Cr: YSGG laser 2780nm wavelength, diode laser 940nm wavelength and sodium hypochlorite 5.25% solution on *E. faecalis* biofilm.

**Materials and Methods****Selection of the samples:**

Fifty extracted human permanent maxillary central incisors were obtained from the outpatient clinic of Oral Surgery Department, Misr International University, Egypt, for the use in this study. All selected teeth were intact and exhibited mature apices with no obvious fractures or cracks. Each tooth was radiographed bucco-lingually and mesio-distally to exclude any tooth with internal resorption or calcification.

**Preparation of the samples:**

Teeth were cleaned and scaled to remove any surface deposits and/or calculus. All Teeth were decapitated at the level of cement-enamel junction using isomet saw under water coolant. The length of all roots was

adjusted to 16 mm by coronal surface grinding. The roots were left for 30 minutes in 5.25% sodium hypochlorite for surface disinfection and soft tissue dissolution and then were stored in tap water to prevent dehydration.

#### **Root canal instrumentation:**

An endodontic explorer was used to locate the orifice of the root canal. The irrigating solution that was used along all the procedural steps for all samples was 5.25% sodium hypochlorite solution, using a 27-gauge needle, two mL between each file size. The root canals were enlarged using ProTaper NEXT (Maillefer, Dentsply, USA) rotary nickel-titanium instruments according to the manufacturer instructions. The canals were prepared to size X4. The apex of each root was sealed with flowable composite resin to guard against bacterial leakage. The roots were mounted vertically in plaster blocks which were numbered, autoclaved for 15min at 121°C to ensure decontamination and stored in normal saline solution at 4°C until application.

#### **Bacterial inoculation for biofilm formation:**

Clinical isolates of *E. faecalis* from the Microbiology laboratory (Central laboratories, Ministry of Health, Egypt) were applied for biofilm formation. The bacterial strain was inoculated in Brain Heart Infusion broth (BHI; Difco Laboratories, Detroit, MI, USA) and incubated at 37°C for one day. The suspension was prepared by cultivating the biological marker on the surface of Brain Heart Infusion agar (BHIA; Difco Laboratories) and incubated at 37°C for one day. The bacterial cells were re-suspended in normal saline solution to prepare a final concentration of about  $3 \times 10^8$  cells/mL. This concentration was adjusted to No. 1 MacFarland turbidity standard then it was applied for contamination of the specimens. The root canals were filled with a one-day pure culture suspension of *E. faecalis*. All

specimens were incubated at 37°C in sealed bottles for 10 days, replacing the intracanal fluids with a fresh normal saline solution, adjusted to No. 1 MacFarland turbidity standard every 72 hours.

SEM examination was used to assess *E. faecalis* biofilm on the root canals after 10 days. Three random specimens were divided into two equal halves using a hammer and chisel as mentioned by Sen *et al.* (13). Each half was fixed in 2.5% glutaraldehyde, pH 7.4, at 4°C for one day, then washed with PBS for 15 min, and postfixed for 12h at 4-6°C in 1% (wt/vol) osmium tetroxide. PBS was applied as a final wash. Dehydration was carried with an ascending acetone series (30%, 60%, and 100%) for 10 min each. The specimens were dried with a SAMDRI PVT-3 critical point dryer (Tousimis Research Corp., Rockville, MD) using liquid CO<sub>2</sub> replacement. Each specimen was mounted and coated with a 200 Å layer of gold palladium. Canal observations were performed by a JEOL JSM-35CF SEM at 30 kV to ensure the biofilm formation (Figure 1).

#### **Sample grouping:**

Based upon the disinfection method, specimens were grouped as follows: Group A (n=15 teeth): Er, Cr: YSGG laser 2780 nm wavelength (Waterlase iPlus, Biolase, USA), Group B (n=15 teeth): diode laser 940 nm wavelength (Epic, Biolase, USA), and Group C (n=15 teeth): Sodium hypochlorite (NaOCl) 5.25% irrigant. Three teeth were used to confirm the biofilms formation by SEM and two teeth served as negative control in which the root canals remained sterile without contamination and irrigated with physiologic saline to ensure aseptic condition.

#### **Root canal disinfection:**

In groups A and B, the laser was activated then; the optical fiber was introduced into the root canals, moved slowly and spirally to the apex then, it was slowly withdrawn. These

steps were carried out in eight second and represented one lasing cycle. Each specimen was irradiated with four lasing cycles, with 15 seconds of rest between each two successive lasing cycle. The total treatment time was 40 seconds/canal. Neither water nor air cooling was applied with both laser types. Er, Cr: YSGG laser 2780 nm wavelength (Waterlase iPlus, Biolase, USA) was applied with the setting factors showed in Table (1).

Table (1): Setting factors of the used Er, Cr: YSGG laser 2780 nm wavelength

Factors	Setting
Average power	1.5 W
Repetition rate	20 Hz
Mode	H (60 $\mu$ s)
Water	80 %
Air	60 %
Tip used	RFT2
Hand piece	Gold

While diode laser 940 nm wavelength (Epic, Biolase, USA) was applied with the setting factors mentioned in Table (2)

Table (2): Setting factors of the used diode laser 940nm wavelength

Factors	Setting
Average Power	1.5 W
Mode	CW
Tip	Non-initiated, 200 microns (E2)
Hand piece	Surgical

Specimens in group C were irrigated with 5mL of 5.25% NaOCl solution for 2 minutes.

#### Bacterial sampling:

Two samples were taken from each root canal, first sample (S1) was taken before disinfection and the second sample (S2) was taken after the disinfection procedures. The samples were transported in 0.9% sterile saline solution. Sterile paper points #30 (Meta Dental Co., Ltd, Korea) were inserted into the root canal to reach the working length as possible, allowed to saturate then, put in tubes containing one mL of sterile saline solution under complete aseptic conditions.

Each specimen was carefully mixed by vortexed for 30 seconds. Serial 10-fold dilution (1:10, 1:100 and 1:1000) was performed using sterile normal solution. Then 0.1 mL from each dilution was plated on BHI agar, incubated at 37°C for two days, and CFUs per one mL were enumerated. After 48hrs, the plates were checked for bacterial growth. All petri-dishes were divided into 4 quadrants and a marker pen was used to mark the dotted colonies. The *E. faecalis* was observed as white pin point colonies on the BHI agar. Further confirmation was carried out by microscopic after using the gram stain.

#### Bacteriological evaluation:

Visible colonies of *E. faecalis* were enumerated in each petri dish and the number of colonies/plate was multiplied by the corresponding dilution factor and by 10 to determine the total CFUs/ mL of each specimen. Disinfection efficacy of various disinfection procedures was assessed by calculating the percentage of reduction in colony counts (%RCC) prior and posts the disinfection.

The percentage of reduction in CFU was calculated as follow:

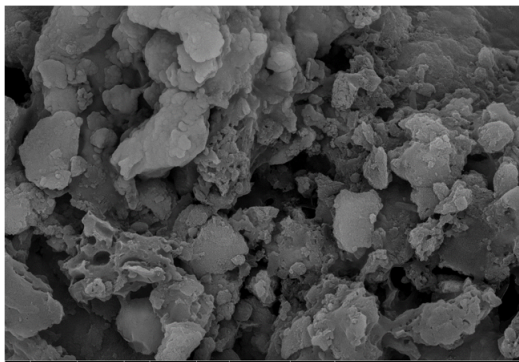
$$\frac{\text{CFU (Before disinfection)} - \text{CFU (After disinfection)}}{\text{CFU (Before disinfection)}} \times 100$$

#### Data collection and statistical analyses:

The data were expressed as mean and standard deviation (SD). As regards Log<sub>10</sub> CFU data, repeated measures ANOVA test was carried out for comparison between the groups. Tukey's post-hoc test was applied for pair-wise to compare between the groups when ANOVA test was significant. The significance level was set at  $P \leq .05$ . Analysis was done by the SPSS program (SPSS INC, Chicago, Il, USA) version 17.0.

## Results :

Absence of *E. faecalis* was observed in the specimens of negative control group. All samples of the three groups showed *E. faecalis* growth before the disinfection. *E. faecalis* was observed as gram +ve cocci arranged in a cross-chain pattern. The strain used suitably colonized around the openings of the dentinal tubules and on the root walls after 10 days of incubation, forming *E. faecalis* mature biofilm in several sites of the root canal, as shown by SEM (Figure 1).



**Figure (1):** A representative scanning electron microscopic image of the developed *E. faecalis* biofilm in the root canal after 10 days of contamination (X 5000).

The number of CFUs and %RCC in the main groups are collected in Table (3). There was no statistically significant difference between the three groups as regard the antibacterial action on *E. faecalis* biofilm ( $P>0.05$ ).

**Table (3):** Log<sub>10</sub> CFU counts in the three groups before and after disinfection and % of reduction in the bacterial counts

Method of disinfection	Time	Mean ± SD	95% CI	
			Lower bound	Upper bound
Er, Cr: YSGG laser 2780 nm wavelength (Group A)	Before irradiation	5.27 ± 0.07	5.23	5.31
	After irradiation	0.94 ± 1	0.38	1.5
	Reduction %	82.88 ± 0.09	82.83	83.92
Diode laser 940nm wavelength (Group B)	Before irradiation	5.26 ± 0.06	5.23	5.29
	After irradiation	1.04 ± 0.51	0.89	1.5
	Reduction %	80.22 ± 0.09	79.85	82.92
NaOCl 5.25% solution (Group C)	Before irrigation	5.27 ± 0.06	5.24	5.3
	After irrigation	0.78 ± 0.03	0.68	0.87
	Reduction %	85.20 ± 0.23	83.55	87.74

## Discussion:

Successful endodontic therapy depends on the root canals' disinfection and establishment of unfavorable conditions for the residual microbes (14,15). Elimination of endodontic infection differs from most other tissues in the human body because the teeth and their root canals have specific anatomy and physiology.

After pulpal infection, microorganisms may penetrate the dentinal tubules and periradicular structures. Chemo-mechanical procedures of root canal preparation are unable to eradicate completely the endodontic infections. About 40-60% of microorganisms in endodontic infections can resist the chemo-mechanical instrumentation (2,3). This may be due to presence of these infections in inaccessible areas inside the root canal system. Moreover, common irrigants applied for disinfection of the root canals during endodontic therapy, such as NaOCl, act through direct contact to microbes. Therefore, bacteria present in deep layers of dentin may resist NaOCl because the penetration power of the irrigator's is limited.

As regards the penetration power of dentine, NaOCl has a limited power (about 130µm) to invade and disinfect the deeper dentinal tissues (16). However, production of laser energy could consider as a method of disinfection of the deep areas within the dentine without direct contact between target and fibre tip (17).

The present study evaluated the antibacterial effect of two laser systems compared with the gold standard NaOCl for endodontic disinfection. Neither Er, Cr:YSGG laser 2780nm wavelength, diode laser 940nm wavelength nor sodium hypochlorite (NaOCl) 5.25% solution, as a sole method of disinfection, was able to eliminate

completely the root canal infection. But the mixture of NaOCl and diode laser (940 nm) has a synergistic bactericidal action as mentioned before (18). Therefore, future studies on different combinations of antibacterial agents and lasers as well as different combinations of lasers are recommended to achieve complete root canal disinfection.

*E. Faecalis* was applied in this study because this microorganism is usually isolated in teeth with persistent infection after endodontic therapy (19). The ability of *E. faecalis* to persist in endodontic infection could be attributed to its ability to resist high concentration of antibacterial agents and wide range of pH as well as its great ability to form biofilms (20).

Although laser has a bactericidal effect, elimination of the root canal system by lasers is problematic because thermal damage to periradicular structure is possible. Therefore it is important to choose suitable settings for each laser type. In addition, a potential bacterial dissemination from the canals to the patients and dental teams through laser smoke is also possible (11).

The current findings revealed that NaOCl disinfection is higher than both laser systems but not significant. This result is in agreement with Le Goff et al. who reported about 85% RCC in CO<sub>2</sub> laser-treated canals while NaOCl disinfection was more than the CO<sub>2</sub> laser disinfection (21).

Er, Cr: YSGG laser has a disinfecting action in the root dentine and its penetration power depending upon its output power however; it has no bacterial specificity (22). In this regard, Gordon *et al.* added that bacterial recovery of *E. faecalis* decreases when the irradiation time or power increases (23). In

contrast to this study in which a 40-second uses of laser, higher disinfection was achieved with a 120-second use of laser than with NaOCl solution (24). On the other hand, Bergmans *et al.* mentioned that the Nd:YAG laser use is not an alternative for some endodontic pathogens *ex vivo* but a possible adjunct to other protocols for endodontic disinfection (25).

The second laser system used in this study was diode laser at 940nm wavelength. Diode laser device is advantageous because it has small dimensions, low cost, power outputs and operating modes (26). We have to take into account this power to avoid the thermal injury of the periodontal ligament and root resorption. Similar diode laser power was used in a previous study (18). In this regard, it is difficult to compare the results of laser disinfection in the previous studies due to differences in wavelength and setting factors (18).

The results of the current study revealed no significant differences between the used procedures for disinfection of the root canal system. This could be explained by the fact that laser light cannot reach all areas with the same effectiveness, similar to NaOCl rinsing. This is in agreement with the results of earlier workers (18). In addition, Benedicenti *et al.* recorded a synergistic antibacterial effect of NaOCl, citric acid and diode laser energy 810 nm wavelength during the root canal disinfection (27).

There are two techniques for using lasers: dry and wet using the fiber in irrigant. The wet technique activates and agitates of the irrigant (laser activated irrigation / LAI) (28). Moreover, four types of lasers (Er:YAG, Er, Cr:YSGG, Nd:YAG and diode laser) have been applied for root canal disinfection (29).

Preethee *et al.* reported superior bactericidal effect of diode laser (908nm) combined with chemical irrigant than diode laser alone during disinfection of the root canal system (30).

Based on the available researches, the conclusion is that laser has bactericidal action but it still cannot alternate the NaOCl solution and it can be applied as a supplement to the present chemical disinfection during RCT. Similar conclusion was previously mentioned (11).

Many future studies are recommended to select the best laser type and its settings, the best method of laser application and the best irrigant used with lasers to obtain more satisfactory disinfection of the root canals during RCT. Moreover, future studies on the laser-induced thermal injury of the periapical tissues are needed.

### Conclusion:

Within the limitations of the current work, both ER, Cr: YSGG laser 2780nm wavelength and diode laser 940nm wavelength have antibacterial efficacy as NaOCl 5.25% solution on *E. faecalis*. The use of laser for root canal disinfection may be advantageous considering several unfavorable actions of NaOCl.

### References

- Jaiswal N, Sinha DJ, Singh UP, Singh K, Jandial UA, Goel S. Evaluation of antibacterial efficacy of chitosan, chlorhexidine, propolis and sodium hypochlorite on *Enterococcus faecalis* biofilm: An *in vitro* study. *J Clin Exp Dent*. 2017; 9:e1066-1074.
- Tennert C, Feldmann K, Haamann E, Al-Ahmad A, Follo M, Wrbas K, Hellwig E, Altenburger MJ. Effect of photodynamic therapy (PDT) on *Enterococcus faecalis* biofilm in experimental primary and secondary endodontic infections. *BMC Oral Health* 2014; 14, Article number: 132.
- Mohammadi Z, Jafarzadeh H, Shalavi S, Sahebalam R, Kinoshita JI. Laser-based disinfection of the root canal system: An update. *J Contemp Dent Pract*. 2017; 18(1):74-77.
- Gejan E, Aghazadeh M, Abshov R, Milani A, Mossavi Z. Microbial flora of root canals of pulpally-infected teeth: *Enterococcus faecalis* a prevalent species. *J Dent Res Dent Clin Dent Prospect*. 2009; 24-27.
- Mohammadi Z. Effects of root canal irrigants on the planktonic form of *Enterococcus faecalis*: A review. *Niger J Med*. 2015; 24(3):261-267 .
- Ozbek S, Ozbek A, Erdorgan A. Analysis of *Enterococcus faecalis* in samples from Turkish patients with primary endodontic infections and failed endodontic treatment by real-time PCR SYBR green method. *J Appl Oral Sci*. 2009; 17(5): 370–374.
- Prabhakar J, Senthilkumar M, Priya MS, Mahalakshmi K, Sehgal PK, Sukumaran VG. Evaluation of antimicrobial efficacy of herbal alternatives (Triphala and green tea polyphenols), MTAD, and 5% sodium hypochlorite against *Enterococcus faecalis* biofilm formed on tooth substrate: an *in vitro* study. *J Endod*. 2010; 36(1):83-6. doi: 10.1016/j.joen.2009.09.040.
- Jones DS, Gorman SP, Mc Cafferty DF, Woolfson AD. The effects of three non-antibiotic, antimicrobial agents on the surface hydrophobicity of certain micro-organisms evaluated by different methods. *J Applied Bacteriol*. 1991; 71: 218-227.
- Tyagi SP, Sinha DJ, Garg P, Singh UP, Mishra CC, Nagpal R. Comparison of antimicrobial efficacy of propolis, *Morinda citrifolia*, *Azadirachta indica* (Neem) and 5% sodium hypochlorite on *Candida albicans* biofilm formed on tooth substrate: An *in-vitro* study. *J Conserv Dent*. 2013; 16(6):532-535. doi: 10.4103/0972-0707.120973.
- Yadav P, Chaudhary S, Saxena RK, Talwar S, Yadav S. Evaluation of antimicrobial and antifungal efficacy of Chitosan as endodontic irrigant against *Enterococcus faecalis* and *Candida albicans* biofilm formed on tooth substrate. *J Clin Exp Dent*. 2017 9(3):e361-e367. doi: 10.4317/jced.53210.
- Kimura Y, Wilder-Smith P, Matsumoto K. Laser in endodontics: A review. *Int Endod J*. 2000; 33: 173-185. Sen BH, Piskin B, Demirci T. Observation of bacteria and fungi in infected root canals and dentinal tubules by SEM. *Endod Dent Traumatol*. 1995; 11: 6-9.
- Mohammadi Z. Laser applications in endodontics: an update review. *Int Dent J*. 2009;

- 59: 35-46.
13. Sen BH, Piskin B, Demirci T. Observation of bacteria and fungi in infected root canals and dentinal tubules by SEM. *Endod Dent Traumatol.* 1995; 11: 6-9.
  14. El-Tayeb MM, Abu-Seida AM, El Ashry SH, El-Hady SA. Evaluation of antibacterial activity of propolis on regenerative potential of necrotic immature permanent teeth in dogs. *BMC Oral Health.* 2019;19:174.
  15. Abdelsalam N, Abu-Seida AM, Fayyad D, Tawfik H. Radiographic and histopathologic outcomes of immature dog teeth with apical periodontitis after revascularization using propolis. An *in vivo* study. *Saudi Endod J.* 2020;10:199-207.
  16. Berutti E, Marini A, Angerreti A. Penetration ability of different irrigants into dentinal tubules. *J Endod.* 1997 23: 725-727.
  17. Moshonov J, Orstavik D, Yamauchi S, Pettiette M, Trope M. Nd:YAG laser irradiation in root canal disinfection. *Endod Dent Traumatol.* 1995; 11: 220-224.
  18. Castelo-Baz P, Martín-Biedma B, Ruíz-Piñón M, Rivas-Mundiña B, Bahillo J, Perez-Estévez A, Gude F, De Moor R, Varela-Patiño P. Combined sodium hypochlorite and 940 nm diode laser treatment against mature E. Faecalis biofilms in vitro. *J Lasers Med Sci.* 2012; 3(3):116-121.
  19. Sedgley CM, Lennan SL, Clewell DB. Prevalence, phenotype and genotype of oral Enterococci. *Oral Microbiol Immunol.* 2004;19: 95-101.
  20. Portenier I, Tuomos MT, Waltimo M, Haapasalo MP. *Enterococcus Faecalis*-the root canal survivor and 'star' in post-treatment disease. *Endod Top.* 2003; 6:135-159.
  21. Le Goff A, Dautel-Morazin A, Guingard M, Vulcain, JM, Bonnaure-Mallet M. An evaluation of the CO2 laser for endodontic disinfection. *J Endod.* 1999; 25: 105-108.
  22. Schoop U, Goharkhay K, Klimscha J, Zagler M, Wernisch J, Georgopoulos A, Sperr W, Moritz A. The use of the erbium, chromium: yttrium-scandium-gallium-garnet laser in endodontic treatment: the results of an *in vitro* study. *J Am Dent Assoc.* 2007; 138: 949-955.
  23. Gordon W, Atabakhsh VA, Meza F, Doms A, Nissan R, Rizoio I, Stevens RH. The antimicrobial efficacy of the erbium, chromium: yttrium-scandium-gallium-garnet laser with radial emitting tips on root canal dentin walls infected with *Enterococcus faecalis*. *J Am Dent Assoc.* 2007; 138: 992-1002.
  24. Asnaashari M, Safavi N. Disinfection of contaminated canals by different laser wavelengths, while performing root canal therapy. *Lasers Med Sci.* 2013; 4 (1): 8-16.
  25. Bergmans L, Mitsiadis P, Teughels W, Van Meerbeek B, Quirynen M, Lambrechts B. Bactericidal effect of Nd:YAG laser irradiation on some endodontic pathogens *ex vivo*. *Int Endod J.* 2006; 39: 547-557.
  26. Marchesan MA, Brugnera-Junior A, Ozorio JE, Pécora JD, Sousa-Neto MD. Effect of 980-nanometer diode laser on root canal permeability after dentin treatment with different chemical solutions. *J Endod.* 2008; 34:721-724.
  27. Benedicenti S, Cassanelli C, Signore A, Ravera G, Angiero F. Decontamination of root canals with the gallium-aluminum-arsenide laser: an *in vitro* study. *Photomed Laser Surg.* 2008; 26:367-370.
  28. De Moor RJG, Meire M. High-power lasers in endodontics - Fiber placement for laser-enhanced endodontics: in the canal or at the orifice? *J Laser Health Acad.* 2014; (1): 20-28. [www.laserandhealth.com](http://www.laserandhealth.com)
  29. Jurič IB, Anić I. The use of lasers in disinfection and cleanliness of root canals: A review. *Acta Stomatol Croat.* 2014; 48(1): 6-15. doi: 10.15644/asc48/1/1
- Preethee T, Kandaswamy D, Arathi G, Hannah R. Bactericidal effect of the 908 nm diode laser on *Enterococcus faecalis* in infected root canals. *J Conserv Dent.* 2012; 15: 46-50.