

Recovery Rate of *E. faecalis* After Er,Cr:YSGG Laser Disinfection of Root Canals: an Ex- Vivo Study

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Abstract **Aim:** This study was carried out to assess the recovery rate of *Enterococcus faecalis* count after disinfection of the root canals with Er,Cr:YSGG laser. **Methodology:** Freshly extracted 40 human teeth of single straight root canals were prepared to a size 50; these specimens were sterilized and then inoculated with *E. faecalis* suspension and incubated for 48 hrs. They were randomly allocated to three treatments and one control negative group. The infected root canals underwent laser treatment. Then samples were collected immediately (after irradiation of root canal) and after 7 days of irradiation, after that surviving of bacteria were checked by counting the colony-forming units (CFU). **Results:** The results indicated that the Er,Cr:YSGG laser was capable of significant reduction of *E. faecalis* in the infected root canal at all selected output powers 0.75W, 1W and 1.5W as well as there was significant antibacterial differences among these powers. In addition the results showed increased in the number of bacterial colonies in the second microbiological sampling compared with the first microbiological sampling, The bacterial recovery decreased when laser irradiation power increased. The best result for the laser that showing less number of *E. faecalis* after 7 days of irradiation at 1.5W. **Conclusions:** This study recognized the disinfection ability of Er,Cr:YSGG laser against the most resistant bacteria that is associated with endodontic infection.

Keywords: *Er,Cr:YSGG laser, E. faecalis, recovery of bacteria, endodontics, bacteriology*

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1. Introduction

Root canal therapy is one of the endodontic branches that deals with the diseases related to the pulp of tooth and its surrounding tissues. Treatments of the infected root canals lead to preservation of natural teeth as a result of function and appearance of the patient's masticating system [1], and aims to eliminate bacteria from infected root canal system and to prevent spreading of infections to the head and neck region [2].

Maintenance of bacteria within the root canal can be considered the principal etiologic factor for the development of pulp and periapical lesions [3]. Different types of microorganisms such as bacteria, fungi, and probably viruses can infect dental pulp and lead to periodontitis. Additionally, spread of infection to the surrounding area of the root significantly reduces the treatment success. Therefore, microorganisms have a major role in the treatment failure of the teeth roots [4]. Thus, success rate of endodontic therapy depends on the probable presence of bacteria. In vital teeth where the number of bacteria in the canal is low, success rate is high. In teeth with lesions around the roots and necrotic teeth, success rate decreases due to the high presence of microorganisms inside the canal [5].

After biomechanical preparation, resistant microorganisms may remain inside the root canals, or even penetrate in the root canal system during or after the endodontic treatment. These microorganisms and their byproducts are able to promote persistent or secondary infections, leading to treatment failure. In these situations, micro-organisms in the root canal is composed of fewer species than primary infections and Gram-positive bacteria predominate [6]. Furthermore, Gram-positive facultative anaerobes bacteria, seem to be remarkably resistant to local antimicrobial agents that are often used in root canal therapy [2].

E. faecalis has high prevalence in root-filled teeth associated with periapical lesions. As well as it possesses several virulence factors that contribute to its ability of surviving the effects of conventional root canal therapy [7]. Prevalence of this microorganism is 22% to 77% of cases [8]. Because of its ability to sustain PH=11/5 and resist calcium hydroxide which is disposed in the canals between treatment sessions. *E. faecalis* can stay alive while starving for a long time and grow alone without support from coexistence with other bacteria in treated canals [9]. As well as, this bacteria is able to invade dentinal tubules and bind to collagen [10]. Thus, dentinal tubules infection by bacteria can act as a source for endodontic recurrent infection. Therefore, disinfection of infected canals is more complicated [11].

Chemomechanical preparation can be conventionally accepted for effective root canal disinfection [12]. Thus, routine irrigation may remove the majority of the bacteria within the canal but may not be very effective to bacteria located deeply within the dentinal tubules [13]. Intracanal medication used to supplement this procedure [14]. However, the effectiveness of these conventional strategies against microorganisms remains under debate [15]. Thus there is the need for new substances and equipment like lasers [16].

Lasers have been suggested to assist endodontic treatments to achieve deep root canal disinfection [17]. It can eliminate microorganisms existing in main canal, lateral canals and dentinal tubules which may cause pulp and periapical infection [16]. Erbium, Chromium: Yttrium-Scandium-Gallium garnet (Er,Cr:YSGG) laser at wavelength 2,780nm, acts through photoablation since this wavelength correlates with the absorption maximum of hydroxyapatite. When irradiated, water contained in the dental hard tissue evaporates instantaneously and ablates the surrounding tissue with only minimal thermal side effects [18]. Although this laser used primarily for the preparation of dental hard substances, it also can be used in treatment of root canals of teeth [19], and disinfection of root canal system without being hazardous to surrounding structures [20]. Recently, in order to improve light distribution inside root canals, modified radial firing tips were developed [13] allowing irradiation of narrow or curved root canals [21].

Finally, success of root canal treatment depends on complete sterilization of root canals. This objective becomes more difficult to achieve as root canal system is polymicrobial in nature. Among the various microorganisms which are responsible for the failure of the root canal treatment, *E.faecalis* holds an important position and is also used as a biological marker [22].

2. Materials and Methods

Forty single-rooted, human teeth were selected and cleaned with curettes to remove calculus and rests of periodontal tissue. The teeth were cut at the cementoenamel junction with a diamond sectioning disc (Germany). Working length was determined by passing a size 15 K-file (Japan) in the canal until the apex was visible. The root canals were enlarged 1 mm short of the root apex (14mm) up to a size 50 K-file under irrigation with physiological saline. The apical foramen was sealed by composite resin (USA) and the root surface covered with bonding agent to prevent bacterial leakage (Figure 1). Roots were then embedded in silicone impression material, covered with aluminum foil, fixed in stainless steel boxes and sterilized by autoclaving (121°C, 15 min).



Figure 1. Prepared roots

2.1. Incubation Conditions

Overnight broth culture (grown at 37°C) of *E.faecalis* in brain-heart infusion (BHI) broth was prepared (4×10^7 cfu/ml) [23] and used to infect the prepared root canals.

2.2. Infection of Root Canals

The sterilized tooth specimens were inoculated with 10 μ L of an overnight BHI broth culture of *E.faecalis* by using a micropipette with a sterile tip. Specimens were kept at 37°C for 42 hour to allow bacterial growth. Three root canals not infected and not treated were control negative. After incubation, the samples were divided into three groups, consisting of 10 specimens each.

2.3. Irradiation of the Root Canals

The specimens were irradiated with an Er,Cr:YSGG laser, $\lambda=2.78\mu\text{m}$ (Figure 2), with radially emitting firing laser tip type RFT3 inside the canals (Figure 3) that mounted on a 90°-angled handpiece. The laser was adjusted for an average output powers 0.75W, 1W and 1.5 W, 20Hz, 10% air flow without water. For each sample, before each irradiation, the tip was disinfected with 70% alcohol. Each sample was treated with one lasing cycle, which comprised five irradiations of 5s duration with a 20s break in between [24]. The tip was placed at the working length (14mm in length). Then, the laser was activated, and the root canal was continuously radiated from apical to coronal, in slow, circling movements. By means of this procedure the irradiation of the entire root canal could be ensured.



Figure 2. Er,Cr:YSGG Laser



Figure 3. Laser tip RFT3 mounted on a 90°- angled handpiece

2.4. Sampling Procedure from Infected Canals

The microbiological procedures were performed under a septic conditions. Immediately after treatment, the sample was taken using sterilized no.1 Gates-Glidden bur to allow dentinal filings [13], then 10mg of dentine chips transferred into vial contain 1ml normal saline. Followed by spreading of 200-µL of this solution on Enterococcus agar plates, incubated overnight at 37°C, then counted the resulting colonies. After that, the root canal openings were covered with wax and the specimens were incubated at 37°C for 7 days. Throughout this period, sterile BHI broth was added to the canals every 2 days. Seven teeth did not receive bacterial suspension, only sterile BHI broth (negative control group-NC). Seven days after inoculation, microbiological sample was performed using sterilized no. 1 Gates-Glidden bur, then transferred the sample to test tubes containing 1 ml of sterile saline solution as mentioned above followed by counting the number of bacterial colony.

3. Results

Bacteriological test showed that absence of growth was seen in the negative controls. All specimens of the three groups showed bacterial growth prior to the experiment disinfection procedures.

Statistical analysis showed that the antibacterial effect of laser at different powers and selected time was highly significant compared with the untreated group using ANOVA at level ($p < 0.001$), as shown in (Table 1) and (Figure 4, Figure 5).

From the cfu counts of bacteria immediately after irradiation for all specimens, the reduction value was observed at 0.75W, 1W, 1.5W of output powers that were significantly different from the number of bacterial colonies before root canals irradiation with laser. Additionally, the result showed that significant antibacterial difference between the output powers that used in this study as the output power increase the antibacterial effect of laser against *E.faecalis* also increase. The result improved complete eradication of *E.faecalis* at irradiation power 1.5W when the sample was taken immediately after irradiation.

When comparison was made between the first(immediately) and the second (after 7 days) microbiological sampling, using ANOVA at level ($p < 0.001$), the results revealed that at all selected output powers there was increased in the number of bacterial colonies after 7 days of laser application in the root canals.

Further comparison from the second microbiological sampling among different output powers, the result indicated that there were significant differences between 0.75W, 1W and 1.5W of output powers in the number of colonies. As well as non significant differences was obtained between the number of *E.faecalis* counts at 0.75W immediately after irradiation of root canal and 1W after 7 days of root canal irradiation. The results are showed in (Table 1) and (Figure 4).

Table 1. One way analysis of variance for the comparison among different output powers of laser and comparison between the recovery of *E.faecalis* counts immediately and after seven days of root canal irradiation

Power(Watt)	No.	T.	Mean	SD	F-value	P-value	Duncan
0.75	10	Immediately	128.2	7.78	664.705	0.000	C
		After 7 days	181.3	17.90			D
1	10	Immediately	44.30	14.78			B
		After 7 days	132.00	7.73			C
1.5	10	Immediately	0.00	0.00			A
		After 7 days	10.50	3.43			A
Before Treatment	10		278.60	19.82			E

According to ANOVA significant different exist at $p < 0.001$, means with different letters vertically have significant difference at $p < 0.001$ according to Duncan test.

SD= Standard deviation.

T= Time of sampling procedure.

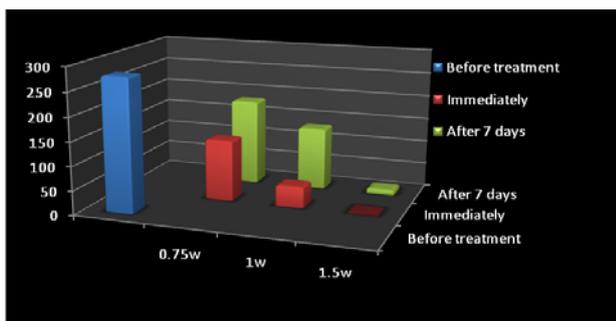


Figure 4. Bar chart showing the antibacterial effect of laser on the *E.faecalis* immediately and after 7 days of irradiation

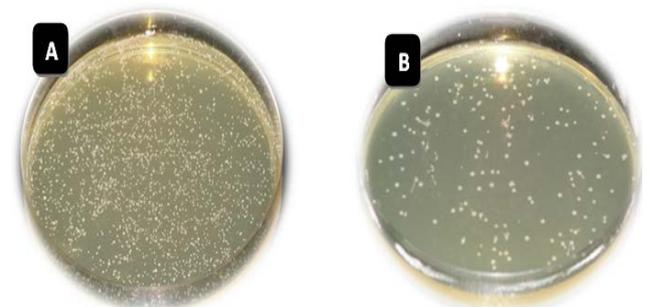


Figure 5. Number of bacterial colonies grown on Enterococcus agar (A) before and (B) after laser irradiation

4. Discussion

Successful endodontology relies, to a great extent, on complete cleaning of the root canal because infected dentine and pulpal tissue can endanger therapy outcome [24]. Bacterial colonization on pulpal disease and endodontic treatment failures is a well-known fact [25]. These bacteria constitute an essential source for the potential reinfection of an endodontically treated root canal [26]. Consequently the complete removal of the pathogenic bacteria and their toxic byproducts is of crucial importance for the therapy outcome [27].

E. faecalis is a Gram-positive microorganism commonly detected in asymptomatic, persistent endodontic infections. Its ability to compete with other microorganisms, invade dentinal tubules and resist nutritional deprivation [7]. It was chosen as the microbiological marker because it has the ability to colonize the root canal in biofilms, representing the *in vivo* growth condition [28]. In addition, it often survives chemomechanical preparation [29] because of its resistance to antimicrobial agents and its ability to cause a monoinfection in the root canals [30]. Moreover, it is resistant to temperature and physical stress [31]. The presence of this microorganism has been related to persistent apical periodontitis [32] and also with failure of endodontical treatments [33], and therefore, is qualified for this investigation.

Conventional root canal treatment aims at the removal of the infected pulp and dentin layers by using mechanical techniques and bactericidal irrigants. However, these cleansing techniques are only successful to a certain extent [27].

Many complications during root canal irrigation, including inadvertent injection of sodium hypochlorite or hydrogen peroxide into the periapical tissues, air emphysema, and allergic reactions to the solutions [34]. Additionally, the discrepancy of the penetration depth of micro-organisms and bactericidal rinsing solutions often holds responsible for therapy resistant cases and long-term failures which can be observed in conventional endodontics [27]. Many studies have demonstrated that prognosis of endodontic treatment in the teeth with periapical lesions are the same as teeth without any lesions if the root canal therapy carried out to an optimal level [35].

Endodontic treatment is costly procedure and for that reason many studies were done to discover new materials and/or methods for the most important step during root canal therapy which was responsible for reinfection of endodontically treated teeth. In this study, we evaluated the ability of an Er,Cr:YSGG laser with radially emitting laser tips to eliminate *E. faecalis* from the infected specimens.

Through the introduction of a new radial-firing fiber tip, the mode of light emission in the root canal has been improved. Owing to the conical shape of the fiber tip, the laser light is emitted in the form of a broad cone with an angle of about 60°, allowing a more uniform coverage of the whole dentinal surface [24].

The results in this experiment showed reduction in the number of *E. faecalis* counts at all output powers when the samples were taken immediately after the Er,Cr:YSGG laser irradiation. This is comparable with others Schoop et al., 2004 [27], Gordon et al., 2007 [13], Eldeniz et al., [36] and René Franzen, 2009 [37].

The bactericidal effect of laser radiation with a wavelength of 2.78µm is based on the high absorption constant for OH⁻ groups and H₂O. The laser destroys *E. faecalis* when energy is absorbed into the volume of the bacterium [37]. Another explanation for the Er,Cr:YSGG laser's positive effect on *E. faecalis* could be some degree of conduction of the laser light within the dentinal tubules, resulting in a penetration of laser light inside it. Other factors, such as shock waves or cavitations effects that have been reported [38].

A different results was achieved Jha and colleagues [39] who concluded that the Er,Cr:YSGG laser was not able to eliminate an *E. faecalis* infection in root canals and that the laser was completely ineffective in disinfecting root canals. The difference in the results may be attributed to differences in the methodology used in the studies.

The result of this study showed significant antibacterial differences between the output powers which is in accordance with Schoop et al., 2004 [27] who showed that laser at a wave length 2.78µm at a power of 1W, generated minor changes in the bacterial count. Increasing the power to 1.5W showed slightly improved bacterial reduction. Similar finding obtaining by Ulrich Schoop, 2007 [21] showed and concluded that Er,Cr:YSGG laser was effective in eliminating the gram-positive *E. faecalis*, at output power of 1 W. When the laser at an output power setting of 1.5W, the result showed no significant difference when compared with the 1 W group. Additionally, study by Wang et al., 2007 [40] showed an experiment in straight root canals that were inoculated with *E. faecalis* for 3 weeks found that the Er,Cr:YSGG laser irradiation resulted in a reduction in bacteria of 77% after irradiation at 1W, and a reduction of 96% after irradiation at 1.5W, but there was no significant difference. Moreover, microbiological reduction of *E. faecalis* was also discussed by René Franzen, 2009 [37] and showed reduction of *E. faecalis* with a minimal laser output power of 0.25 W.

The result of this experiment showed that the antibacterial effect of Er,Cr:YSGG laser is dependent on the output power as the output power increase the effect against *E. faecalis* also increase. Different studies showed similar results [13,27].

The basic research study showed statistically significant bactericidal effect on *E. faecalis* with 2.780nm radiation when the sample was taken immediately after laser application in the root canals, where as in the second microbiological sampling after seven days of irradiation the results showed increased in the number of *E. faecalis* counts at 0.75W, 1W and 1.5W of output powers in one lasing cycle at the selected time.

One possible explanation for the increased number of bacterial colony counts after seven days of irradiation is the ability of the remaining viable *E. faecalis* that was not exposed to the irradiation to grow rapidly. This could be due to availability of sufficient nutrition in the root canal. Although the group that is exposed to 1.5W showed negative result in the first microbiological sampling but after 7 days the result showed the presence of bacteria in the same group this could be due to the presence of viable bacteria deeply inside the dentinal tubules not subjected to irradiation, this may be due to the time used in this experiment was not enough to kill all the bacteria, therefore, need more than one lasing cycle.

The present findings in accordance with the study by Castelo-Baz et al., 2012 [41] who showed that after 7 days, a high microbial growing in laser group. An explanation can be provided by the fact that laser light cannot reach all areas. Furthermore, survival of *E. faecalis* can be attributed to the high resistance of *E. faecalis* to heat, because of its cell-wall structure [27].

According to the results of current study all the selected output powers reduce the number of bacterial colony of *E. faecalis* and the most effective power was 1.5W that a negative result in the first microbiological sampling but due to positive result in the second microbiological more time of laser application is required in order to kill all the bacteria that is penetrated deeply in the dentinal tubules and prevent reinfection in the root canals.

The penetration of the laser into the root dentin is governed by several factors. At the wavelength of the Er,Cr:YSGG laser, there is absorption by dentin owing to the presence of hydroxide and interstitial water. On the basis of the fact that each laser pulse is composed of approximately 150 micropulses and each micropulse is responsible for the penetration of this energy of about 3µm into water, depending on fluence, it is possible to achieve expansion of intratubular water and the collapse of water vapor as deep as 1,000µm or more. This effect, known as "micropulse-induced sequential absorption," with expansion and collapse of water vapor, is capable of producing acoustic waves strong enough to disrupt intratubular bacteria. This penetration of dentin may provide the laser with advantages versus conventional methods of dentin disinfection that has limit antimicrobial activity [13].

E. faecalis has the ability to maintain viability in obturated root canals [42] and because of the fact that, none of the filling materials were able to prevent the regrowth of *E. faecalis* completely [43]. For that reason, more work needs to be done; to achieve the goal of complete elimination of infection from the root canal of teeth and the clinical applications of this strategy are under evaluation in my Department.

5. Conclusions

1. The study concluded that the wavelength tested study is a suitable for eliminating bacteria from the infected root canals.
2. Ability of remaining viable *E. faecalis* to grow rapidly in the root canal after 7 days of irradiation with laser.
3. Time used in this experiment was not enough to eliminate *E. faecalis* completely from the infected root canal. Therefore, more time is required during laser irradiation. For the results to be confirmed further, another clinical studies are necessary.

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