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Antibacterial Effect of Er:YAG Laser Irradiation Applied by a New Side-Firing Spiral Tip on *Enterococcus faecalis* Biofilm in the Tooth Root Canal—An *Ex Vivo* Study

Nathanyel Sebbane [1,2], Doron Steinberg [1,*], David Keinan [3,4], Ronit Vogt Sionov [1], Adi Farber [1,2] and Sharonit Sahar-Helft [2,3]

[1] Biofilm Research Laboratory, Faculty of Dental Medicine, Institute of Dental Sciences, The Hebrew University of Jerusalem, 9112102 Jerusalem, Israel
[2] Department of Endodontics, Faculty of Dental Medicine, Hebrew University-Hadassah, 9112102 Jerusalem, Israel
[3] Department of Endodontics, School of Graduate Dentistry, Rambam HCC, 3525408 Haifa, Israel
[4] The Ruth and Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, 3525408 Haifa, Israel
* Correspondence: dorons@ekmd.huji.ac.il; Tel.: +972-52-4681999

Abstract: This study evaluates the antibacterial and anti-biofilm effect of erbium:yttrium aluminum garnet (Er:YAG) laser treatment on a tooth root model infected with *Enterococcus faecalis*. Background: New treatment options are required to overcome endodontic infection in periapical tissue. Studies using Er:YAG during endodontic treatment yielded promising results regarding anti-biofilm/antimicrobial effects. Methods: The root canals of 80 teeth were incubated with *E. faecalis* for 4 weeks, allowing biofilm formation in the root canals, then divided into one control group and seven treatment groups that were exposed to Er:YAG laser using a side-firing spiral Endo tip, 2.5% sodium hypochlorite (NaOCl), 17% EDTA solutions alone or combined. The number of bacteria in each sample was determined by counting the number of colony-forming units (CFU) and was statistically compared. Results: Er:YAG laser, NaOCl and EDTA treatments alone caused a 76.0 ± 5.7%, 98.0 ± 0.6% and 69.0 ± 9.1% reduction, respectively, in CFU. Combining the laser treatment with NaOCl, EDTA or both, caused a further reduction in the bacterial load by 99.77 ± 0.14%, 93.4 ± 1.6% and 99.95 ± 0.04%, respectively. Conclusions: Er:YAG laser treatment showed significant antibacterial effect on the experimental groups, while combination with NaOCl and EDTA provided the most efficient conditions for achieving antibacterial effect against *E. faecalis*, in the tooth root model.

Keywords: Antibacterial; Bacteria; Biofilm; EDTA; *E. faecalis;* Irrigation solution; Er:YAG laser; NaOCl; Side-firing spiral Endo tip

Citation: Sebbane, N.; Steinberg, D.; Keinan, D.; Sionov, R.V.; Farber, A.; Sahar-Helft, S. Antibacterial Effect of Er:YAG Laser Irradiation Applied by a New Side-Firing Spiral Tip on *Enterococcus faecalis* Biofilm in the Tooth Root Canal—An *Ex Vivo* Study. *Appl. Sci.* **2022**, *12*, 12656. https://doi.org/10.3390/app122412656

Academic Editors: Sun-Young Kim and Ji-Hyun Jang

Received: 15 November 2022
Accepted: 7 December 2022
Published: 9 December 2022

1. Introduction

Endodontic treatment aims to reduce the amount of microorganisms inside the root canal system in order to heal or prevent lesions of endodontic origin. Endodontic procedures involve chemo-mechanical treatment of the root canal system with irrigations to improve debridement and disinfection. The complex anatomy and microstructure of the root canals hinder the mechanical cleaning to reach all the canal spaces, resulting in microbes left behind [1]. Thus, the common cleaning procedures are unable to remove all the biofilm-covered surfaces and all the infected regions [2–4].

Biofilms are organized, surface-attached microbial communities embedded in an extracellular polymeric substance (EPS), which protects the microbes from external stress stimuli [5]. Mixed microbial biofilms can appear within the dentinal tubules and root ca-

nal spaces, which require interventions [6]. The Gram-positive *Enterococcus faecalis* bacterium has been related to the failure of root canal treatment since the bacteria may survive the endodontic procedures and penetrate the dentinal tubules up to 800 microns [6].

Biomechanical preparation of the tooth root is accomplished by irrigation solution. The most commonly used solution in endodontic treatment is sodium hypochlorite (NaOCl) which is well-known to be highly potent at killing bacteria [7] and causes organic tissue dissolution [8]. However, NaOCl has unwanted side effects that can result in ecchymosis, tissue necrosis and paresthesia [9]. Another commonly used irrigation solution contains EDTA that chelates calcium ions, resulting in the removal of the mineralized portion of the smear layer [10]. Previous studies have shown that alternate irrigation with EDTA and NaOCl improve the removal of the infected region [11].

Since the conventional irrigation techniques have the limitation of not reaching all parts of the root canal as a result of its complex anatomy, new strategies with improved physiochemical properties have been developed. Already in 1994, Oguntebi, B.R. [12] suggested that it would be preferable to develop a local treatment technique with anti-microbial activities that can reach the dentinal tubules, rather than using the systemic antibiotic approach. Laser technology presents a safe and efficient treatment for eradicating bacteria and removing the infected regions from root canals [13,14]. The Er:YAG laser technique has become popular especially after its usage on teeth has been approval by the United States Food and Drug Administration (FDA) [13]. Importantly, the Er:YAG laser has a bactericidal activity against several bacterial species [15,16].

According to Sahar-Helft et al. [17], improved anti-bacterial and anti-biofilm effects can be reached by increasing the Er:YAG laser energy–effective power (W), and by using a larger size tip, or by being in closer proximity to the biofilm. In this study, the Sapphire tip was used; however, this tip has several limitations. Using this technique, the emission of the laser beam is directed along the root canal and not necessarily laterally along the root canal walls. Thus, it is almost impossible to obtain a uniform 360-degree coverage of the internal aspect of the root canals [18]. In the present study, we propose to use a new side-firing spiral Endo tip to increase the coverage. The Endo tip is specially designed for cleaning and disinfecting the root canals during endodontic treatment and endodontic retreatment (Figure 1). The tip has a hollow, flexible, conical, and round cross-section with circumferential spiral slits throughout its length which fits the shape and volume of the processed root canals.

The treatment is based on the delivery of Er:YAG laser light which is highly absorptive in water at approximately 1–3 μm penetration depth [19]. The laser energy causes a cavitation bubbles effect which results in a movement much deeper in the main root canal than the traditional methods of a syringe or ultrasonic waves [20]. The irrigation absorbed by the Er:YAG laser energy has not only an effect on the lateral canals and other outlying structures, but also on the apical part of the root canal [19]. Laser-activated irrigation permits a major cleaning mechanism by liquid velocity resulting from the laser-induced cavitation bubble effect [21].

Endodontic therapy includes removal of the microflora and irritants from the canal and the periapical tissues, and thorough debridement is essential for the treatment to be successful [22]. Success of root canal treatment depends on complete eradication of biofilms and their byproducts from the root canal system [23]. Endodontic treatments include various irrigants as an adjunct along with mechanical preparation for the disinfection of the root canals. Their major goal is to remove the bacterial layer that has penetrated inside the root canal and to prevent reinfection.

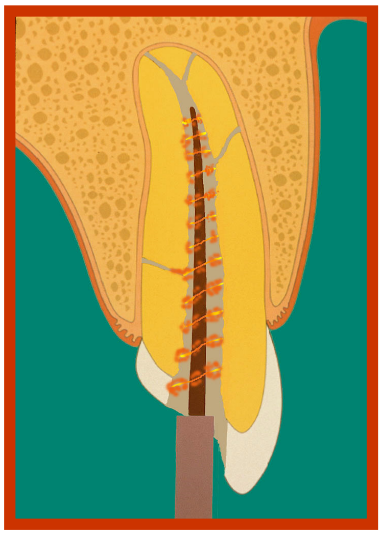

Figure 1. The image illustrates the novel Endo tip (in dark brown color) inside a root canal, where the propagation of the laser energy is all along the canal, including the lateral walls. The image was kindly obtained by Prof. Adam Stabholz at our Faculty.

The aim of this research was to study the efficiency of the combined treatment of alternate NaOCl and EDTA irrigations with Er:YAG laser exposure applied by the Endo tip in reducing the *E. faecalis* bacteria load in biofilms formed on tooth root canal walls.

2. Materials and Methods

2.1. Tooth Preparation

Eighty root canals of extracted human teeth [24] were used in the study (Helsinki number 040617-HMO). The teeth were extensively washed in double-distilled water (ddw), sterilized when being soaked in ddw using a 121 °C steam autoclave for 20 min and then stored in saline at 4 °C until use. The teeth were prepared with No. 2 and 3 gates Glidden drills for coronal flaring, a 10 k-type file until the apical foramen was visible, and the working length was determined by subtracting 1 mm from this length. Then, a 15 k-type file, a 20 k-type file, and ProTaper Next (Dentsply Maillefer, Baillaigues, Switzerland) rotatory files to x3 was used. Finally, 35 k-type and 40 k-type files were used to create an effective chemical-mechanical cleaning. Between files, the canals were irrigated using 10 mL of water through a 27-gauge needle and patency was maintained using a 10 k-type file. After finishing the canal preparation, the teeth were decoronated using a straight handpiece with a disc to obtain uniform root canal length. The teeth were divided into uniform groups and soaked in 25 mL of 70% ethanol for 2 h to create neutralized laboratory conditions, remove previous material residues, and then washed ten times with 50 mL sterile ddw.

2.2. Root Canal Infection

The prepared teeth were incubated with *E. faecalis* ATCC 29212 bacteria for 28 days in an anaerobic environment at 37 °C. The bacteria were obtained from a −80 °C stock. The initial bacterial culture was prepared by incubating 100 µL of the bacteria stock in 10 mL of brain heart infusion broth (BHI; Acumedia, Lansing, MI, USA) for 16–18 h at 37 °C. Ten teeth of each treatment group were cultured with the bacteria in 30 mL BHI supplemented with 1% glucose in 50 mL tubes. The initial bacteria suspension had an $OD_{600\ nm}$ of 0.1. Two-thirds of the medium was replaced every two days by fresh medium. The bacterial cultures were regularly inspected under a light microscope to verify that there is no contamination by other bacterial species. At the end of the incubation period, the teeth were washed three times with 30 mL phosphate-buffered saline (PBS).

2.3. Laser Specifications

The Er:YAG laser (Light Instruments, Yokne'am, Israel) was applied using the Endo tip—a side-firing spiral tip (Light Instruments, Yokne'am, Israel) with a circumferential spiral slit that is hollow, flexible, conical and round cross-section. Specifications: 1.5 W:150 mJ, 10 Hz, duration 60 s.

2.4. Treatment

After 28 day incubation with the bacteria and three washes in PBS, the teeth were divided into 8 groups containing 10 teeth each as follows:

Group 1: Infected teeth only.
Group 2: Infected teeth irrigated with 10 mL of a 2.5% NaOCl solution for 60 s.
Group 3: Infected teeth irrigated with an Er:YAG laser-Endo tip and 10 mL of a 17% EDTA solution for 60 s.
Group 4: Infected teeth irrigated with an Er:YAG laser-Endo tip and 10 mL of a 2.5% NaOCl solution for 60 s.
Group 5: Infected teeth irrigated with an Er:YAG laser-Endo tip and 10 mL of saline (0.9% NaCl) for 60 s.
Group 6: Infected teeth irrigated with 10 mL of a 17% EDTA solution for 60 s.
Group 7: Infected teeth irrigated with 10 mL saline for 60 s.
Group 8: Infected teeth irrigated with an Er:YAG laser-Endo tip and 10 mL of a 17% EDTA solution for 60 s, following irrigation with 10 mL of a 2.5% NaOCl solution for 60 s.

The canals were irrigated according to each group's plan in a sterile hood environment, using a sterile syringe and needle. 2.5% NaOCl solution was prepared by diluting 6% NaOCl (Romical, Israel) in sterile ddw. DSI 17% EDTA solution was obtained from Dentech, Israel.

2.5. Determining the Number of Live Bacteria in the Root Canal

Following the different treatments, the bacteria in the root canals were collected using a Hedstrom file (Dentsply) size 45 k, filed for 16 sec circumferentially at the buccal, lingual, mesial and distal areas of the teeth. The Hedstrom files were each separated from a plastic handle using a cutter and placed in an Eppendorf tube containing 30 µL of BHI broth. All samples were then vortexed for 5 s and diluted thousand and ten thousand fold times in BHI. 20 µL of the diluted samples were spread on BHI agar plates using Drigalski spatulas and the plates were incubated at 37 °C for 24 h. Each agar plate was photographed and magnified, while the CFU [25] was counted using the ImageJ software. The number of live bacteria in the tooth root was calculated by multiplying the CFU with the dilution factor and corrected for the volume.

2.6. Statistical Analysis

Each treatment was performed on eight teeth. The Student's T-test in the Microsoft Excel program with ad hoc corrections was used to analyze the statistical significance of the differences between the various treatment groups. A p-value of 0.05 or less was considered statistically significant. The data obtained from the individual tooth root samples are presented.

3. Results

3.1. Comparison of the Anti-Bacterial Effect of 2.5% NaOCl with and without Er:YAG Laser Treatment on E. faecalis Biofilm in Tooth Root Canals

In the first series of experiments, we wanted to know if Er:YAG laser treatment following 2.5% NaOCl irrigation provides a better anti-bacterial effect than 2.5% NaOCl irrigation alone. To this end, *E. faecalis* biofilms were allowed to be formed in the tooth root

canal prior to treatment for 28 days. Thereafter, the root canals were washed with 2.5% NaOCl solution for 60 s with or without Er:YAG laser treatment. In addition, the last group of infected root cannels were treated in the serial order of 17% EDTA solution, NaOCl solution and Er:YAG laser exposure. Whereas irrigation with NaOCl caused a 98.0 ± 0.6% reduction in the bacterial count ($p < 0.01$; Figure 2), the combined treatment with Er:YAG laser caused an even stronger reduction of 99.77 ± 0.14% ($p < 0.01$ in comparison to control; Figure 2). Exposing the root canals to 17% EDTA prior to NaOCl and Er:YAG laser treatment, caused even a further reduction in CFU to 99.95 ± 0.04% (with $p < 0.01$ in comparison to control; Figure 2). These data demonstrate the importance of adding an Er:YAG laser-Endo tip to the root treatment with NaOCl and EDTA.

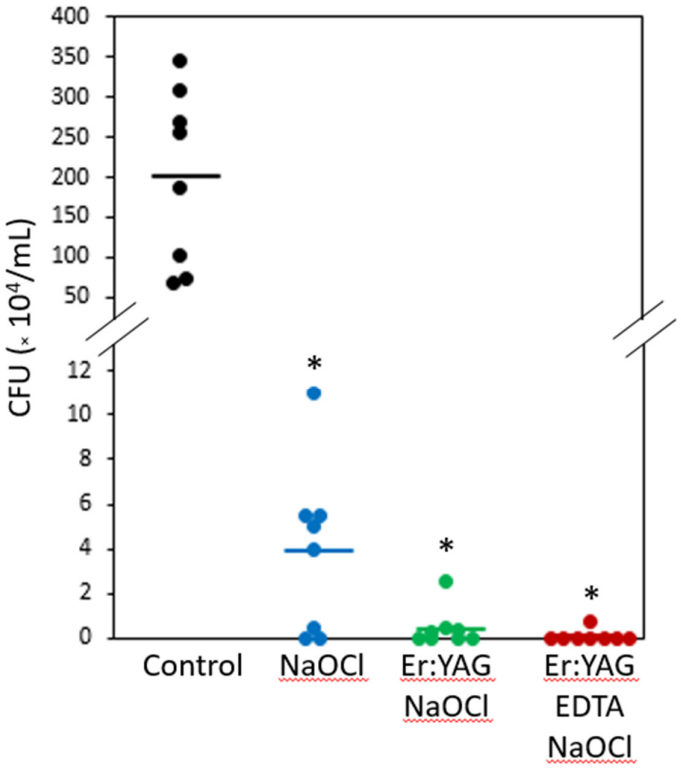

Figure 2. Comparison of the anti-bacterial effect of Er:YAG laser–Endo tip alone or combined with 2.5% NaOCl. The number of *E. faecalis* colonies (×10⁴ CFU/mL) observed 24 h after treatment of the infected teeth using the following protocols: Group 1: The CFU of *E. faecalis* in the tooth root canal without treatment (Control; black). Group 2: The CFU of *E. faecalis* after 2.5% NaOCl irrigation (blue). Group 4: The CFU of *E. faecalis* after treatment with the Er:YAG laser-Endo tip combined with 2.5% NaOCl irrigation (green). Group 8: The CFU of *E. faecalis* after treatment with the Er:YAG laser-Endo tip combined with 17% EDTA irrigation and 2.5% NaOCl irrigation (red). N = 8 in each group. * $p < 0.01$ for treated samples compared to control. $p < 0.02$ for Er:YAG with NaOCl versus NaOCl alone. No significant difference ($p = 0.28$) between Er:YAG with NaOCl and Er:YAG with NaOCl and EDTA.

3.2. Comparison of the Anti-Bacterial Effect of EDTA Solution with and without Er:YAG Laser Treatment on E. faecalis Biofilm in Tooth Root Canals

Next, we compared the effect of adding Er:YAG laser treatment to root canals irrigated with 17% EDTA. The average value of colonies in the group treated with EDTA irrigation (blue) decreased by 69.0 ± 9.1% when compared to the control group (black) that was only infected by the bacteria without treatment ($p < 0.01$; Figure 3). In the group treated with an Er:YAG laser-Endo tip and a 17% EDTA irrigation (green) and the group

treated with an Er:YAG laser-Endo tip and 17% EDTA following a 2.5% NaOCl irrigation (red), the CFU was reduced by 93.4 ± 1.61% and 99.95 ± 0.04%, respectively, when compared to untreated infected root samples ($p < 0.01$; Figure 3).

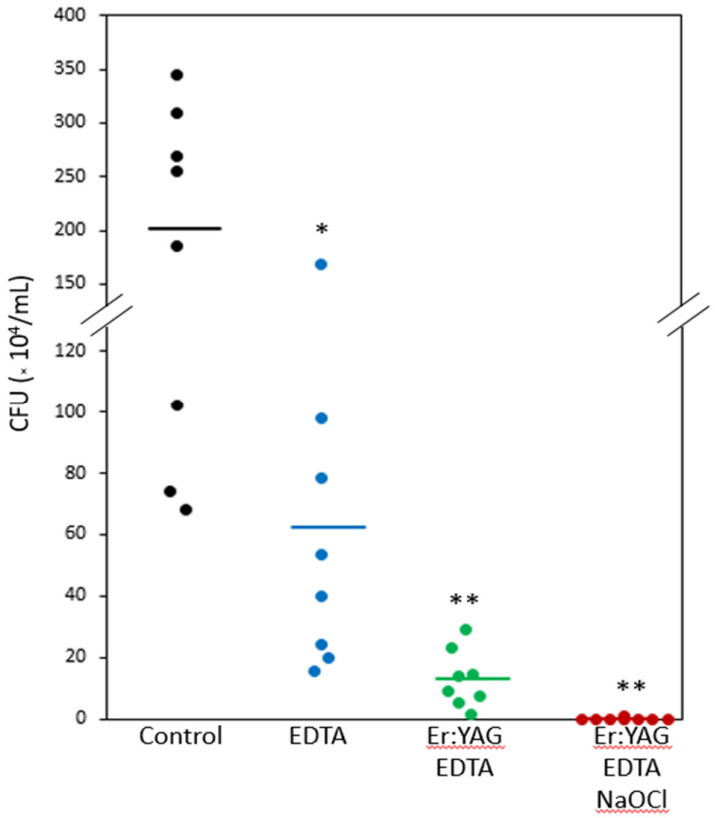

Figure 3. The number of *E. faecalis* colonies after treatment of infected teeth was compared between control (untreated infected root canals) and 3 groups of 17% EDTA irrigation: Group 1: The CFU of control samples infected with *E. faecalis* without receiving any treatment (black). Group 6: The CFU of *E. faecalis* in the root canals after a 17% EDTA irrigation (blue). Group 3: The CFU of *E. faecalis* after using an Er:YAG laser-Endo tip and a 17% EDTA irrigation (green). Group 8: The CFU of *E. faecalis* after using an Er:YAG laser-Endo tip and a 17% EDTA irrigation, followed by a 2.5% NaOCl irrigation (red). N = 8 in each group. * $p < 0.01$ for treated samples compared to control. ** $p < 0.001$ for treated samples compared to control. $p < 0.02$ for EDTA versus ER:YAG laser with EDTA. $p < 0.001$ for ER:YAG laser with EDTA and ER:YAG laser with EDTA and NaOCl.

3.3. Comparison of the Anti-Bacterial Effect of Er:YAG Laser–Endo Tip Combined with NaOCl and/or EDTA Irrigation of Infected Teeth on E. faecalis Biofilm in Tooth Root Canals

Here, we wanted to compare the effect of Er:YAG laser-Endo tip treatment alone and in combination with various irrigation combinations of NaOCl and EDTA. The average number of *E. faecalis* colonies in the group treated with an Er:YAG laser-Endo tip and saline irrigation decreased by 76.01 ± 5.71% compared to the control untreated infected samples ($p > 0.01$; Figure 4). In the samples treated with an Er:YAG laser-Endo tip and a 17% EDTA irrigation step, the CFU was reduced by 93.44 ± 1.61% in comparison to that of the control group ($p > 0.01$; Figure 4), this treatment was more efficient than the Er:YAG laser-Endo tip and saline irrigation group, but this was not statistically significant ($p = 0.12$; Figure 4). The group treated with an Er:YAG laser-Endo tip and a 2.5% NaOCl irrigation, the CFU was reduced more significantly than Er:YAG laser-Endo tip and saline irrigation ($p < 0.001$; Figure 4). The CFU of the samples treated with an Er:YAG laser-Endo tip and a

17% EDTA following a 2.5% NaOCl irrigation decreased even more than Er:YAG laser-Endo tip and a EDTA irrigation ($p < 0.001$; Figure 4).

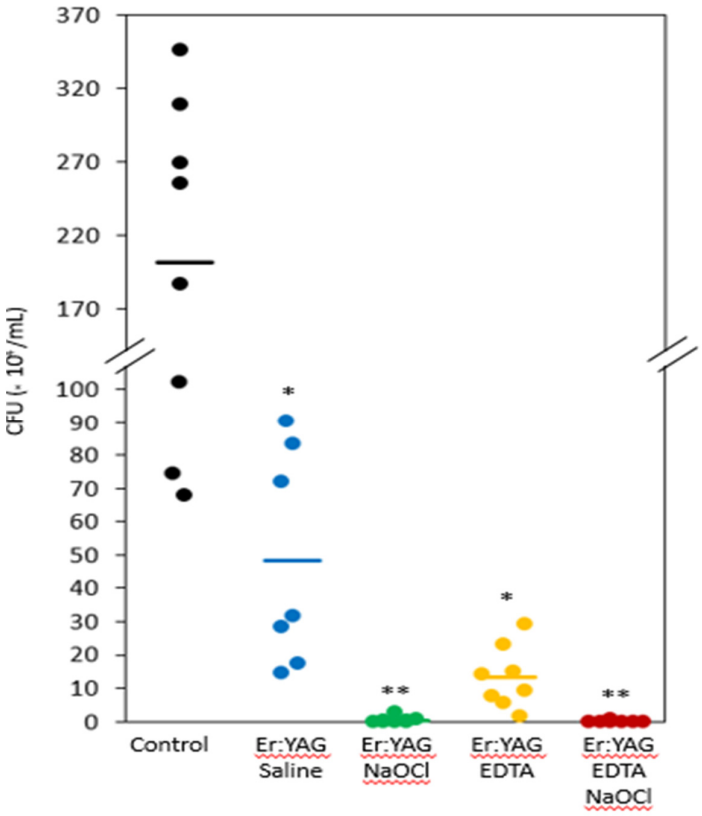

Figure 4. The number of *E. faecalis* colonies after treatment on infected teeth with Er:YAG laser-Endo tip and different irrigation solutions in comparison to the control group. Group 1: The CFU of *E. faecalis* in control infected samples without treatment (black). Group 5: The CFU of *E. faecalis* after using an Er:YAG laser-Endo tip and saline (blue). Group 4: The CFU of *E. faecalis* after using an Er:YAG laser-Endo tip and a 2.5% NaOCl irrigation (green). Group 3: The CFU of *E. faecalis* after using an Er:YAG laser-Endo tip and a 17% EDTA irrigation (yellow). Group 8: The CFU of *E. faecalis* after using an Er:YAG laser-Endo tip and a 17% EDTA irrigation, followed by a 2.5% NaOCl irrigation (red). N = 8 in each group. * $p < 0.01$ for treated samples compared to control. ** $p < 0.001$ for treated samples compared to control.

3.4. Er:YAG Laser-Endo Tip Treatment Alone Had Only a Partial Anti-Bacterial Effect on E. faecalis Biofilm in Tooth Root Canals

It was also important to study the effect of Er:YAG laser-Endo tip treatment alone on the bacterial load of *E. faecalis* in the infected root canals. Therefore, we compared the CFU of remaining bacteria in control, saline irrigated and Er:YAG laser-Endo tip-saline irrigated root canals. The average of colonies in the saline irrigation group decreased from 2.25×10^6 CFU/mL to 1.5×10^6 CFU/mL compared to the control untreated infected group (Figure 5), however, this decrease was not statistically significant ($p = 0.50$). In the group treated with an Er:YAG laser-Endo tip and saline irrigation, the bacterial load was reduced to 1.25×10^6 CFU/mL, which is a less bacterial burden compared to control infected samples, ($p = 0.01$; Figure 5), but not significant when compared to the saline irrigated samples ($p = 0.13$; Figure 5). It is clear that neither treatment group was effective alone in significantly reducing the bacterial load, although the Er:YAG laser-Endo tip protocol did have a certain anti-bacterial effect on *E. faecalis* biofilms.

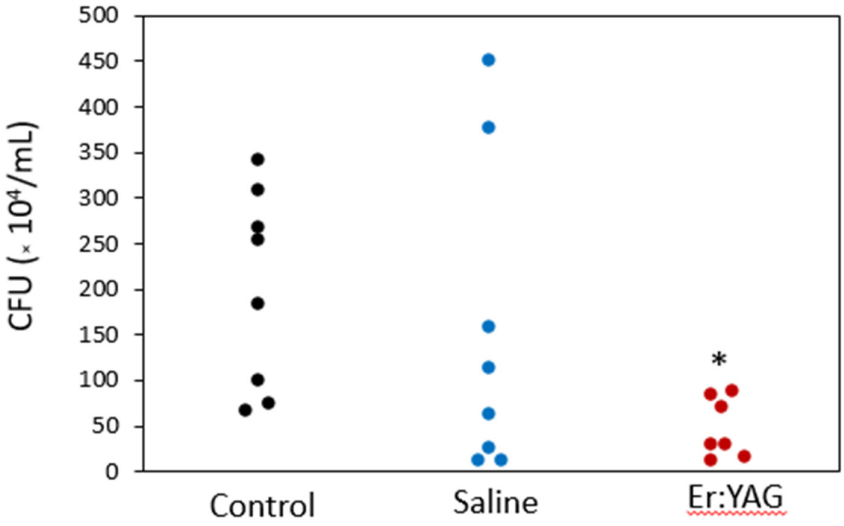

Figure 5. The number of colonies after treatment on *E. faecalis*-infected teeth with Er:YAG laser-Endo tip. Group 1: The CFU of *E. faecalis* in control infected samples without treatment (black). Group 7: The CFU of *E. faecalis* after saline irrigation (blue). Group 5: The CFU of *E. faecalis* after using an Er:YAG laser-Endo tip and saline irrigation (red). N = 8 in each group. * $p < 0.01$ for treated samples compared to control.

4. Discussion

It is well established that endodontic treatment aims to shape and clean the root canal system to heal or prevent periapical disease [26]. This goal can be mainly achieved by significantly decreasing the bacterial inoculum inside the root canal system and preventing re-infection [27]. Although primary endodontic infection is mainly caused by a polymicrobial infection that is predominantly anaerobic, secondary endodontic infection is characterized by fewer strains and is especially facultative [28]. *E. faecalis* is the most common isolated bacteria strain from failed endodontic cases [28,29]. Therefore, numerous studies have used *E. faecalis* as a gold standard for comparing residual infection after different treatment techniques and materials [7,30]. Yet, the main limitation of these studies is that they cannot be fully standardized due to variations in canal size and microanatomy. Furthermore, *E. faecalis* infection can be very challenging to eliminate due to its high resistance to medicaments used during root canal treatment and its survival capability [29,31].

Clinically, various recommended solutions and techniques have been offered to disinfect the root canal system [26]. Among them, the most commonly used solutions are NaOCl and EDTA. All agents used in this study are in clinical use for endodontic treatments. Long term use of NaOCl in the root canal can lead to adverse effects. However, in our study it was used for only 60 s, which does not cause any damage to the tooth [32]. EDTA may also be cytotoxic if applied for long periods, while its short application for 60s as done in our study, does not have any significant side effects on the tooth [33]. A conventional syringe is the basic way to deliver the irrigation solution inside the root canal [34]. The disadvantage of this method is that it has little effect on the apical area [35] or inside the dentinal tubules [36]. As a result, several techniques have been tested to improve the efficiency of those solutions [37]. However, in most cases, it was found that microorganisms inside the complex root canal system can escape the disinfection effect of these solutions due to their ability to penetrate the dentinal tubules. Furthermore, it was also found that biofilm is highly resistant to different antibacterial agents. In the last decade, many studies have been conducted to investigate the effect of the Er:YAG laser with

different solutions and techniques to disinfect the root canal system [38,39]. It was observed that using an Er,Cr:YSGG laser irradiation with 3% NaOCl inhibited the growth of *E. faecalis* and provided complete elimination of the bacteria in all canals. Our previous study found that using an Er:YAG laser with 17% EDTA produced the best results for removing the smear layer [24]. This approach allowed the irrigation solution to reach the open dentinal tubule, enhancing its killing effect on the *E. faecalis* biofilm.

Laser-activated irrigation, with its new side-firing spiral and Endo tip, significantly enhances the effectiveness of root canal disinfection by cavitation effect [40]. The new Endo Tip was specially designed to fit the shape and volume of root canals after they were prepared. This design allows the Er:YAG laser beam to be delivered to the canal walls and its effectiveness can also be improved by adding a final rinse with NaOCl [38,41]. The circumferential pattern is due to the Endo tip's special design that includes a flexibility zone (3 mm) with slits that allow the laser beam to be emitted laterally directly towards the walls of the root canal and generate rapid agitation of fluid [42]. The wavelength of the Er:YAG laser (2940 nm) is close to the absorption peak of water (3000 nm) [43], such that the energy of Er:YAG laser is maximally absorbed by the water-based irrigation solutions upon irradiation. This leads to explosive boiling irrigation, induction of cavitation bubbles in close proximity to canal walls during their collapses, generation of shear flows that are able to remove particles from the surface. These vapor bubbles create a volumetric expansion of 1600 times the original volume [44], which drives fluid along the root canal in a manner that improves smear-layer removal from the root-canal wall [45]. DeVito et al. [46] described and patented a procedure called Photon-Induced Photoacoustic Streaming (PIPS) for removing the smear layer and disinfecting root canals using an Er:YAG laser. Our previous study showed that irradiating closer to the biofilm induces a collateral bactericidal effect and reduces bacterial viability within the biofilm [17]. Lukac et al. [47] recently introduced shock wave-enhanced emission photoacoustic streaming (SWEEPS). Yang et al. [48] described special laser tips placed only in the pulp chamber and advocated for cleaning while minimally shaping the root canal system. The results of the antimicrobial effect of the PIPS method in comparison to laser-activated irrigation are inconsistent [49].

The presents study was carried out on an *ex vivo* model for evaluating microbial infection in the root dentin based on the model first introduced by Haapasalo and Orstavik [50]. We found that combining laser activation with either 17% EDTA or 2.5% NaOCl resulted in a statistically significant decrease in the number of bacterial colonies ($p < 0.01$). The best antibacterial effect was achieved by combining the Er:YAG laser-Endo tip with 17% EDTA followed by 2.5% NaOCl. This remaining CFU number using the Er:YAG laser-Endo tip was between 4% and 0.0004% when combined with EDTA alone or with both EDTA and NaOCl, respectively. Moreover, the Er:YAG laser-Endo tip treatment significantly affected the CFU even when being used with only saline in the absence of any antiseptic solution ($p < 0.01$).

The influence of Er:YAG laser light absorbed by NaOCl is very effective. The effect is likely caused by a physical mechanism. The Er:YAG laser light generates violent shock waves with greater liquid displacement. The most intense fluid motions are located near the fiber tip [51]. According to Sahar-Helft et al. [17], irradiating at closer proximity to the biofilm was found to significantly reduce bacterial viability within the biofilm ($p < 0.05$). This observation explains the reason of using the novel Endo-tip in our research. Using fiber tip deeper inside the root canal resulted in better biofilm removal. The energy provided by the fiber tip is near the walls and along the entire root canal, generating shock waves that contribute to the efficacy of debridement and removal of the biofilm [52].

There are several safety limitations regarding the clinical use of laser energy with a Sapphire or optical fiber tip in intracanal treatment [53]. The energy from the tip is directed along the root canal and not necessarily laterally to the root canal walls [18]. Direct laser irradiation from the tip of the optical fiber in the vicinity of a tooth's apical foramen

may result in the transmission of the laser beam beyond the foramen, which can be dangerous in proximity to the mental foramen or the mandibular nerve. Matsumoto [54] strongly recommended that the endodontic tip be improved to allow irradiation of all areas of the root canal walls.

The rationale of a new Endo tip is to create ideal irradiation inside the root canal system, promote the efficacy of removing the smear layer and have a bacteriocidic effect on bacteria inside the root canal. In our research, the unique Endo tip was used with both a side-firing spiral tip and a sealed tip to prevent the transmission of radiation to or through the apical foramen of the tooth. Furthermore, the Endo tip fits the shape and volume of root canals and radiates directly laterally to the walls of the root canal after root canal preparation. According to our investigation [17], Er:YAG laser tip close to the biofilm showed a significant biofilm reduction. However, laser intensity was not found to significantly affect biofilm reduction. Contrary to the effect of diode laser irradiation on *E. faecalis*, the bactericidal effect of diode lasers was significantly better at 2.5 W compared to 1.0 W, 1.5 W, and 2.0 W [55]. The emitted energy from various laser systems in dentistry can be delivered into the root canal system by an optical fiber (Nd:YAG, Er:YSGG, and diode) or by a hollow tube (CO2 and Er:YAG) that has the ability to have a bactericidal effect and provide additional cleansing and disinfecting of the root canal system following biomechanical instrumentation. Laser-activated irrigation was presented by Blanken et al. and De Moor et al. [44,56].

The treatment involves delivering a laser beam through a tip into the root canal filled with liquid. The energy of the Er:YAG laser is highly absorptive in water at between 1–3 μm penetration depth [19], resulting in explosive boiling, cavitation bubbles that create shock waves in proximity to canal walls during their collapses and generating shear flows capable of removing particles from the surface [57].

Additionally, due to the emitted shock waves in proximity to the canal walls, they encounter the root canal wall at supersonic speeds. With this energy, the technique may be more efficient in cleaning and disinfecting the root canal system.

5. Conclusions

Considering the results, we can draw several conclusions:

- First, irradiated areas showed a significant reduction of *E. faecalis* biofilm in all experimental groups, indicating Er:YAG's capability in inducing a focal bactericidal effect at the target site. This conclusion is in accordance with De Meyer et al. [52];
- Second, the Endo tip is unique in that it has both a side-firing spiral tip and a sealed tip that radiates directly laterally to the walls of the root canal;
- Third, we found that using an Er:YAG laser beam with either EDTA, NaOCl or even saline had an antibacterial effect on *E. faecalis*. The best antibacterial effect was achieved in the group treated with the Er:YAG laser beam with EDTA and then a final rinse with NaOCl.

Author Contributions: Data curation: N.S., A.F.; formal analysis: N.S., A.F.; investigation: N.S., A.F.; resources: S.S.-H., D.S.; supervision: D.S., S.S.-H., R.V.S.; writing—original draft: N.S., A.F.; writing—review and editing: D.K., D.S., S.S.-H., R.V.S., N.S. All authors have read and agreed to the published version of the manuscript.

Funding: This study was partially funded by the CABAKOFF FOUNDATION, grant number 3175006340.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank Adam Stabholz for authorizing us to use "Figure 1".

Conflicts of Interest: The authors declare no conflict of interest.

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