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ORIGINAL ARTICLE

The in vitro efficacy of biofilm removal from titanium surfaces using Er:YAG laser: Comparison of treatment protocols and ablation parameters

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Abstract

Background: The aims of the present study were to compare the antibacterial effect of Er:YAG laser with other acceptable decontamination methods and to single out the optimal laser device parameters for effective bacterial elimination.

Methods: A multispecies biofilm which was composed of *Streptococcus sanguis*, *Actinomyces naeslundii*, *Porphyromonas gingivalis,* and *Fusobacterium nucleatum* was grown on sandblasted and acid-etched (SLA, homogeneous moderately microrough, and nanosmooth surface) titanium disks. The biofilm was removed from the coated disks by hand curets, ultrasonic device, nylon brush (dental polishing prophy cup), or Er:YAG. Additionally, different parameter combinations of the laser machine were examined to reach an optimal lasing power for bacterial elimination/reduction. Residual biofilm samples were stained with bacterial live/dead staining and quantified using a fluorescent microscope.

Results: A multispecies biofilm was accumulated on the SLA titanium surfaces exhibiting cluster distribution next to bacteria-poor areas. Hand curets, nylon brushes, and the ultrasonic device showed limited capability to effectively remove the biofilm from the SLA surfaces as opposed to the Er:YAG which displayed a superior ability to remove the biofilm. All Er:YAG parameter combinations that were evaluated as well as the tested "tip to target" distances showed similar excellent anti-biofilm effects. Furthermore, we observed that the Er:YAG capability of biofilm removal is not only due to its light emission, but depends on its water irrigation as well.

Conclusions: Er:YAG laser has an excellent biofilm removal capability compared with hand curets, ultrasonic devices, or nylon brushes even when low energy parameters and low power settings are used. Additionally, an excellent antibacterial effect can be reached using a non-contact mode of 1 to 5 mm "tip to target" distance.

KEYWORDS
implantology, lasers, treatment planning

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1 | INTRODUCTION

With the increased number of implants being placed, complications concerning osseointegrated implants arise, including peri-implantitis, which is responsible for most implant losses.

It is acceptable today that microbial colonization on the implant surface is the main causative factor in the pathogenesis of peri-implant inflammation[1–3] which, if untreated, might lead to extensive bone resorption and finally to implant loss. Therefore, the removal of bacterial biofilm seems to be the primary requirement in treating peri-implant infections.[4] This treatment approach actually mimics the strategy adopted for periodontal disease treatment but, unlike periodontal breakdown that happens around living tissues such as cementum and dentin, peri-implant inflammation develops next to non-biological metal surface. Moreover, the screw-type structure of most exposed implants and the microstructured surfaces used to enhance osseointegration makes it almost impossible to effectively decontaminate the implant surface; additionally, existing methods have been unable to reach the apically located implant surfaces in the narrow vertical bone defects that characterize bone anatomy in peri-implantitis cases. Consequently, effective implant decontamination is almost impossible to achieve which explains the poor prognosis of treatment.

In search for an effective bacterial killing tool, laser technology was found to be promising. The unique bactericidal potential of all dental lasers makes them an excellent tool for decontaminating implant surfaces. Among dental lasers, the 2.94 μm Er:YAG laser, has been mostly investigated for its application in periodontal and peri-implant therapy.[5–10] In vitro studies have shown that Er:YAG laser can achieve almost complete elimination of surface bacteria on contaminated titanium surfaces.[11]

To understand the specific interaction that occurs between different laser devices and the treated tissue or element that is affected by its beam, several concepts should be clarified:

1. Each laser wavelength affects specifically the various components (chromophores) of the target tissue: its water content, vascularity, chemical composition, tissue color, etc. This is the basic determinant of the laser-tissue interaction. Thus, the two main characteristics that provide the basis for laser-tissue interaction are the laser's wavelength and the optical features of the target tissue and both are constant.
2. The power of the laser emission (Watts) is determined by multiplying the pulse energy (mJouls) and the frequency of pulse repetition (pulse per second measured in Hertz).
3. Pulse width (mSec)measures the amount of time during which the laser beam is allowed to strike the target tissue each time and has an effect on the rate of tissue temperature rise. Some lasers have a pre-set pulse width that cannot be changed.
4. Spot size is the diameter of laser spot on tissue creates a certain amount of energy per square millimeter of tissue and is referred to as energy density or fluence. The smaller the spot size, the greater the fluency.
5. The use of water or air spray affects the rate of tissue vaporization and temperature changes during treatment.
6. Beam delivery with or without contact affects the beam's divergence and consequently its fluency; if no contact is applied, the distance of the tip from the target is calculated to estimate the energy per field unit.

Apart from laser wavelength and target tissue characteristics, which are constant, the combination of the abovementioned parameters by the operator has an impact on achieving the desired outcomes. The huge variation between the countless studies that evaluated laser efficacy derives in part from the endless parameter combinations that have been tested. Each group of researchers has been using a particular parameter combination.[10] Consequently, no consensus exists regarding the optimal parameters of Er:YAG laser recommended for maximum bacterial elimination.

Therefore, the aims of this in vitro study were: 1) to compare the antibacterial effect of Er:YAG laser in eliminating bacteria from biofilm-coated SLA titanium disks with standard mechanical decontamination methods that are used 2) To determine the optimal parameter combination for effective bacterial elimination by Er:YAG laser.

2 | MATERIALS AND METHODS

2.1 | Bacteria

F. nucleatum PK1594, *P. gingivalis* ATCC 33277, *S. sanguis* NC02863, and *A. naeslundii* 17233 were grown separately in Wilkins-Chagren broth,[*] and incubated at 37°C for 24 hours, under anaerobic conditions (N_2 85%, H_2 5%, CO_2 10%). *S. sanguis* and *A. naeslundii* were transferred to Wilkins broth enriched with 2% sucrose[†] and cultured under anaerobic conditions for an additional 24 hours. *F. nucleatum* and *P. gingivalis* were transferred to fresh Wilkins broth and incubated for an additional 24 hours under anaerobic conditions. The bacteria were then cen-

[*] Oxoid, Basigstoke, Hampshire, UK
[†] Sigma, Rehovot, Israel

trifuged (4,000 rpm, 15 minutes, room temperature) and suspended in gingival cervical fluid (GCF)-simulating medium[12] (60% RPMI medium, 40% donor horse serum[‡§]) enriched with 5 μg/mL hemin and 0.5 μg/mL menadione.[§] The bacterial suspensions were adjusted spectrophotometrically to 10^9 cells/mL for *S. sanguis, A. naeslundii, and P. gingivalis*, while *F. nucleatum* was adjusted to 10^8 cells/mL.[13–16]

2.2 | Multispecies biofilm[17]

Titanium disks (9-mm diameter and 2-mm thickness) with sandblasted and acid-etched (SLA) surface[**] were washed with phosphate buffered saline (PBS) and a suspension of *Sterp. sanguis* and *A. naeslundii* (1:1 ratio in a total volume of 1,000 μL) was inoculated and incubated for 24 hours at 37°C under anaerobic conditions. The disks with the newly formed biofilm were then washed with PBS, a suspension of *P. gingivalis* and *F. nucleatum* (1:1 ratio in a total volume of 1,000 μL) was inoculated and incubated for an additional 48 hours at 37°C under anaerobic conditions. The mature biofilms were washed and reconstituted in PBS (400 μL/disk).

2.3 | Cleaning protocols

The biofilm-covered disks underwent the following treatments (n = 3 for each group) in an attempt to assess their bactericidal capability: ultrasonic device,[††] hand curet,[‡‡] nylon hand brush, and Er:YAG with various parameter combinations laser.[§§] The treatments with ultrasonic device/hand curet/hand brush were performed with direct vision using magnification lopes (×3.5 magnification[***]) and applying strokes that covered the entire disk area in two perpendicular directions. Er:YAG laser treatment was delivered using a 1.3-mm diameter and 17-mm long sapphire tip for a 10-second duration, with water irrigation of 30 mL/min, focusing the beam at the center of the disk. The laser beam was conveyed with direct vision using specific protective eyeglasses.

In an attempt to acquire the optimal parameters combination for desirable bacterial elimination, we modified the pulse energy (mJ), pulse frequency (Hz), and the tip's distance from target (mm). Combination of the tested parameters is detailed in Table 1.

TABLE 1 Laser parameters combination

Laser parameter Combination	Energy	Frequency	Tip distance
Combination 1	20 mJ	45 Hz	3 mm
	40 mJ	45 Hz	3 mm
	50 mJ	45 Hz	3 mm
Combination 2	40 mJ	40 Hz	3 mm
	40 mJ	45 Hz	3 mm
	40 mJ	50 Hz	3 mm
Combination 3	40 mJ	45 Hz	1 mm
	40 mJ	45 Hz	3 mm
	40 mJ	55 Hz	5 mm

When stated, the laser was used with the exclusion of the mirror inside the hand piece, which is responsible to reflect the laser beam from the machine to the tip. This prevents the laser beam from reaching the tip and thus serves as a control that includes water irrigation without light emission.

2.4 | Fluorescence staining

A fluorescence microscope[†††] with a 1 × 20 or 1 × 400 lens was used to visualize the distribution of live and dead bacteria throughout the biofilm. The live bacteria were observed after SYTO9 staining (LIVE/DEAD BacLight bacterial viability kit[‡‡‡]) and dead bacteria were detected after staining with a 1.0 mg/mL propidium iodide (PI) solution.[§§§] The biofilms were stained in the dark at room temperature for 25 to 30 minutes. Fluorescent intensity of each image was measured using ImageJ software. Results are presented as total bacterial biomass as live and dead staining together in mean fluorescent intensity units. The percentage of live bacteria was calculated as the fluorescent intensity units of the live (SYTO9) staining divided by the fluorescent intensity units of live and dead staining together.

2.5 | Statistical analysis

All experiments were performed in triplicates and repeated at least three times. The data were analyzed with a statistical software package.[****] One-way repeated measure anal-

‡ Biological Industries, Beit Ha'emek, Israel
§ Sigma, Rehovot, Israel
** MIS, Bar-Lev Industrial Zone, Israel
†† Acteon, Merignac, France
‡‡ Gracey 5-6, Hu-Friedy, Chicago, IL
§§ 2940 nm, LiteTouch, Light Instruments, Yokneam, Israel
*** Zeiss, Oberkochen, Germany

††† Olympus FV300, Tokyo, Japan
‡‡‡ Molecular Probes, Eugene, OR
§§§ Sigma, Rehovot, Israel
**** SigmaStat, Jandel Scientific, San Rafael, CA

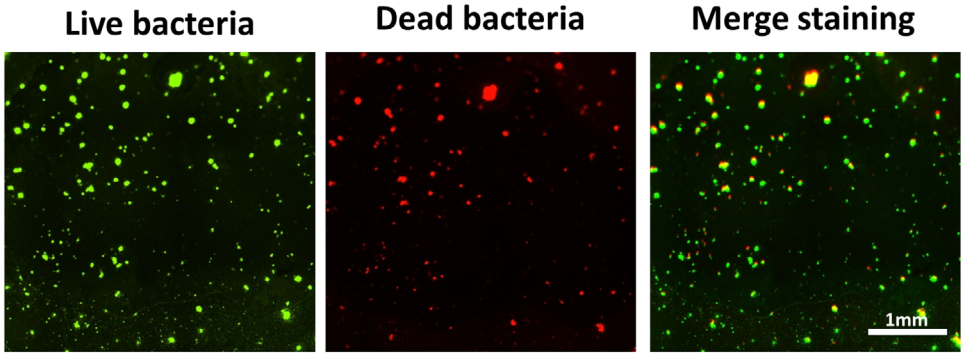

FIGURE 1 Fluorescent microscopic images of biofilm on SLA titanium surface. Multispecies biofilm were stained with live-dead fluorescent dyes and visualized using fluorescent microscopy (magnification ×20). Green staining—live bacteria only; red staining—dead bacteria only; yellow—live and dead bacteria

ysis of variance was applied to test the significance of the differences between the treated groups. If the results were significant, inter-group differences were tested for significance according to Student *t*-test and the Bonferroni correction for multiple testing.

3 | RESULTS

3.1 | Biofilm formation on titanium surfaces

Initially, the study examined the biofilm formation on the SLA titanium disks. The matured multi-species biofilm was stained with live (green) and dead (red) dyes and analyzed under fluorescent microscopy at ×20 magnification.

A representative image depicted a typical biofilm structure on the titanium, which displayed a non-confluent arrangement of bacterial clusters surrounded by areas of poorly dispersed bacteria (merge image, Fig. 1). The majority of the biofilm that composed the clusters contained live bacteria (green staining, Fig. 1) and some clusters contained dead bacteria (red or yellow staining, Fig. 1) as well.

3.2 | Biofilm removal following different clinical disinfection methods

Common clinical biofilm removal techniques were assessed for effectiveness; they included ultrasonic device, hand curet, hand brush, and Er:YAG laser application (energy = 40 mJ; frequency = 45 Hz; energy density: 138.46 J/cm2/pulse; tip distance from target = 3 mm).

A low magnification, which was used to present the overview of live and dead biofilm in Figure 1 (x20), does not allow adequate resolution of differentiation of stained bacterial samples and therefore, a higher magnification (×400) has been chosen for the postoperative evaluation.

Results show that treatment with the ultrasonic device and the hand curets were able to reduce the biofilm mass. However, large islands of viable (green) bacteria were detected following their use (Figs. 2B and 2C compared with control sham treatment, Fig. 2A). Treatment with a nylon hand brush severely increased the dispersal of the biofilm content, resulting in smeared bacterial structure (Fig. 2D). Treatment with Er:YAG laser was the most effective in biofilm removal, leaving almost no detectable viable bacteria on the titanium surface (Fig. 2E).

The quantification of fluorescence in the microscopic images is presented in Figure 3. The bars represent the total values of the biofilm biomass (live and dead) measured as a total fluorescent intensity. Er:YAG laser was significantly more effective in reducing biofilm compared with the control group (Fig. 3A, $P < 0.05$). The least effective treatment was the nylon hand brush which resulted in the highest residual biofilm mass (Fig. 3A, $P < 0.05$). The relative viable biofilm count was increased following hand curets and hand brush treatments, which does not the situation following treatments with ultrasonic or laser devices (Fig. 3B, $P < 0.05$).

3.3 | Effect of Er:YAG laser pulse energy, frequency, and tip distance on biofilm removal

Although the use of Er:YAG laser was the most effective treatment for biofilm removal from titanium surfaces, some biofilm remnants were visible following the laser treatment (Fig. 2E). In the next part of the study, we tested the anti-bacterial activity of the Er:YAG laser using different setting of laser parameter.

Multiple experiments were performed using different parameter combinations of the Er:YAG laser as described below. The parameters that were tested included: 1) pulse energy (tested at 20/40/50mJ); 2)pulse frequency (tested at

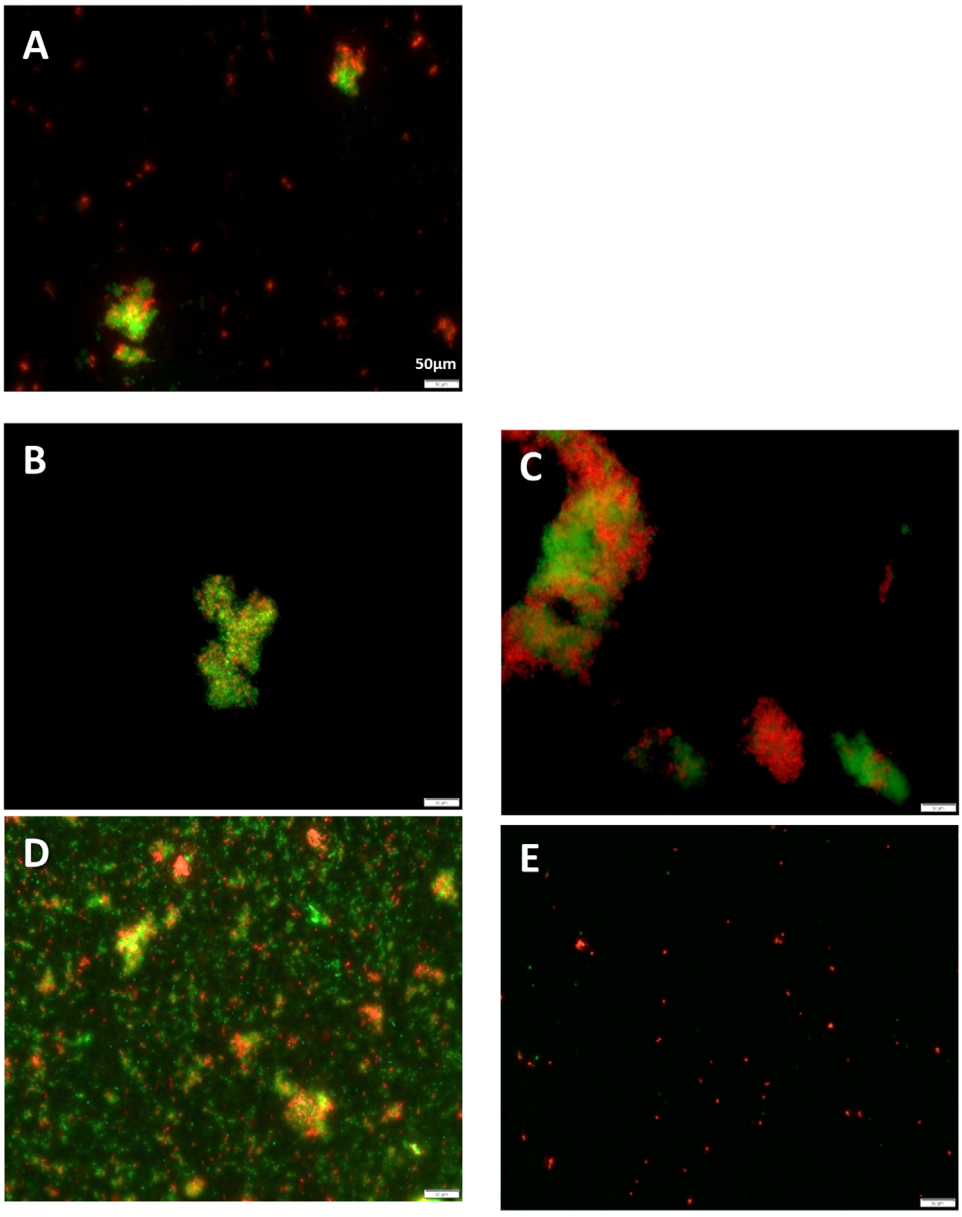

FIGURE 2 Biofilm clearance of SLA surfaces. Multispecies biofilms were cleared using the following methods: **A)** Sham control; **B)** Ultrasonic device; **C)** Hand curets; **D)** Hand brush; and **E)** Er:YAG laser (energy = 40 mJ; frequency = 45 Hz; tip distance from target = 3 mm). The residual biofilm was then stained with live-dead fluorescent dyes and visualized using fluorescent microscopy (magnification ×400). Green staining—live bacteria only; red staining—dead bacteria only; yellow—live and dead bacteria

40/45/50Hrz); and 3) distance of pulse delivery tip from target (tested at 1/3/5 mm).

3.3.1 | Pulse energy

The laser was set at 20/40/50mJ while the pulse frequency (45 Hz) and the distance of the tip from the titanium disk surface (3 mm) were constant resulting in power of 0.9, 1.8, and 2.25 Watts and energy density of 69.12, 138.46, and 173.07 J/cm2/pulse, respectively. All combinations reduced efficiently the biofilm area compared with control (P <0.05), with no difference between the different energies (Fig. 4). No differences in the remaining viable bacteria following laser application were observed between the various parameter combinations (Fig. 4).

3.3.2 | Pulse emission frequency

Frequencies of 40/45/50 Hz were tested in combination with constant energy of 30 mJ and tip-target distance of 3 mm resulting in power of 1.2, 1.35, and 1.5 Watts

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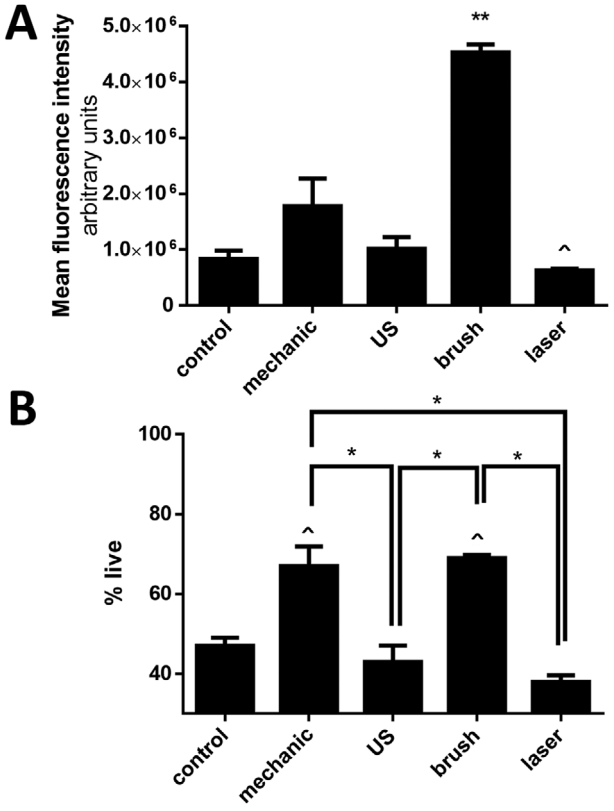

FIGURE 3 Quantification of biofilm clearance. Quantification of total biofilm figures using ImageJ and presented as mean fluorescence intensity in arbitrary units (**A**) and percentage of live bacteria (**B**). Groups included Sham control; ultrasonic (US) device; Hand curets (mechanic); Hand brush (brush); and Er:YAG laser (energy = 40 mJ; frequency = 45 Hz; tip distance from target = 3 mm). *Statistical differences between the marked groups. ^Statistical differences from the control group. **Statistical differences from all other groups

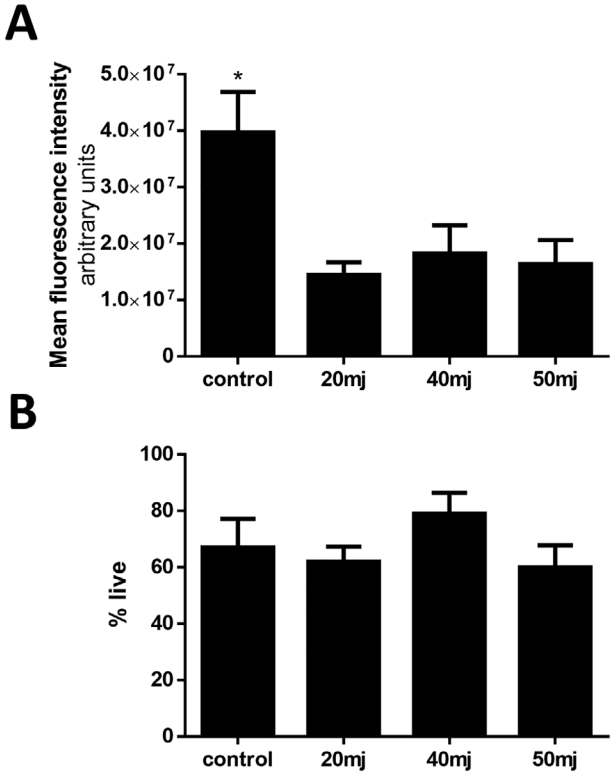

FIGURE 4 Er:YAG pulse energy depended biofilm clearance. Multispecies biofilms were cleared using: Sham control and Er:YAG laser at different pulse energies (20, 40, or 50 mJ) with constant frequency (45 Hz) and tip distance from target (3 mm). Residual biofilm was stained with live-dead fluorescent dyes and visualized using fluorescent microscopy (magnification ×400). Quantification of total biofilm figures using ImageJ and presented as mean fluorescence intensity in arbitrary units (**A**) and percentage of live bacteria (**B**). *Statistical differences from all other groups

and energy density of 92.3, 103.84, and 115.38 J/cm^2/pulse, respectively. Changing the pulse frequency did not significantly affect the bactericidal properties of the laser beam, other than being more effective than sham control (Fig. 5, P <0.05).

3.3.3 | Distance of laser tip from target

A distance of 1/3/5 mm of the laser tip from the disk was tested, while the laser energy and frequency remained constant (30 mJ and 45 Hz respectively (energy density of 103.84 J/cm2/pulse), resulting in 1.35 Watts). Tip-target distance did not affect the bactericidal properties of the laser, despite being significantly more effective than the sham control (Fig. 6, P <0.05). Interestingly, all laser treated groups showed a small but significant increased viability of the biofilm compared with control (Fig. 5, P <0.05).

3.4 | Impact of laser's water spray on biofilm removal

Finally, we examined the contribution of the water spray to the laser's anti-bacterial efficacy by asking ourselves whether the effect of the laser stems only from its light emission or whether its intense water spray has an additional effect. By removing the internal mirror that directs the beam to the tip from the hand piece, we disallowed beam delivery to the target without affecting the water spay. This delivery was compared with a fully functional laser (with the mirror).

The results showed that the exclusion of the laser beam leaving solely the water spray in function resulted in minor biofilm reduction and no change in bacterial viability. Conversely, laser application using both, a functioning beam with a water irrigation, significantly reduced biofilm mass with negligible changes in its viability (see supplementary Fig. S1 in online Journal of Periodontology, P <0.05).

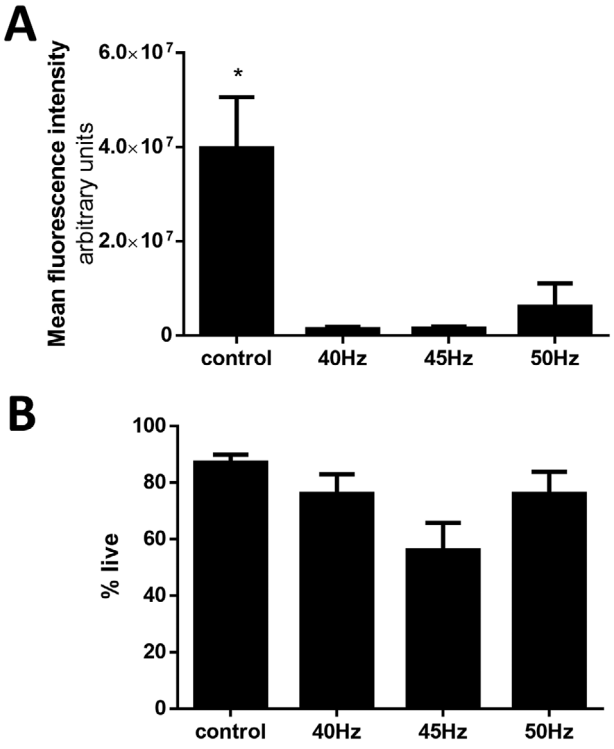

FIGURE 5 Pulse emission frequency depended biofilm clearance. Multispecies biofilms were cleared using: Sham control and Er:YAG laser at different pulse emission frequencies (40, 45, or 50 hz) with constant energy (40 mJ) and tip distance from target (3 mm). Residual biofilm was stained with live-dead fluorescent dyes and visualized using fluorescent microscopy (magnification ×400). Quantification of total biofilm figures using ImageJ and presented as mean fluorescence intensity in arbitrary units (**A**) and percentage of live bacteria (**B**). *Statistical differences from all other groups

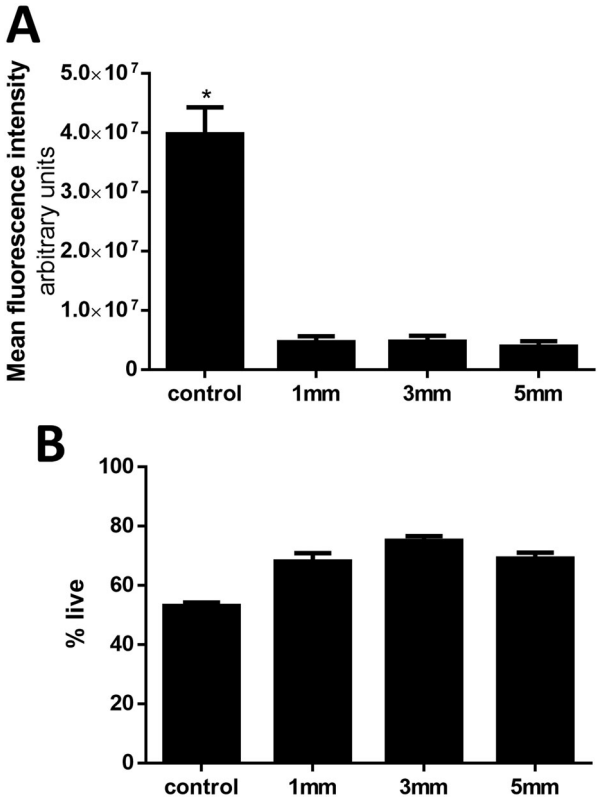

FIGURE 6 Distance of laser tip from target depended biofilm clearance. Multispecies biofilms were cleared using: Sham control and Er:YAG laser at different distances of laser tip from target (1, 3, or 5 mm) with constant energy (40 mJ) and frequency (45 Hz). Residual biofilm was stained with live-dead fluorescent dyes and visualized using fluorescent microscopy (magnification ×400). Quantification of total biofilm figures using ImageJ and presented as mean fluorescence intensity in arbitrary units (**A**) and percentage of live bacteria (**B**). *Statistical differences from all other groups

4 | DISCUSSION

The current study provides additional evidence of the superior efficacy of Er:YAG laser decontamination. This effect exceeded that of conventional anti-infective tested methods. Furthermore, the various parameter combinations of the Er:YAG laser machine that were evaluated displayed similar anti-bacterial effect.

Although the etiology of peri-implantitis is still elusive, it is believed that successful treatment lies in adequate implant surface disinfection. Consequently, the treatment of peri-implantitis mimics that of periodontitis and involves the use of curets, ultrasonic devices, machine-driven brushes, etc. for non-surgical mechanical debridement. However, clinical evidence has shown disappointing outcomes of such treatment[18] and has suggested that flap elevation should be included as well. It is assumed that the ineffective clinical consequences of non-surgical treatment approach stem partly from poor access to hidden implant surface.[19] Therefore, laser technology has been added as an additional tool to the acceptable treatments for better access and improved decontamination.

The first series of experiments focused on the antibacterial effect of the three most popular cleaning methods and compared with that of the Er:YAG laser in vitro; this, however, did not include air ablation which is also a popular treatment modality for peri-implantitis. The results demonstrate a convincing biofilm removal superiority of the Er:YAG laser compared with all other tested modalities. Similar results were published by Eick et al. who showed significantly better biofilm removal from titanium surfaces following Er:YAG application compared with three other treatments (hand curets, photodynamic therapy, and their combination).[20] Eick et al. based their analysis on CFU counts, which is a less sensitive method for quantitative and qualitative analysis since it examines only viable microorganisms. The present study used fluorescent dye-based microscopy, which allows a higher resolution quantification of the biofilm content and a more

accurate biofilm analysis as well as simultaneous evaluation of viable and dead microorganisms. Such evaluating methodology is valuable since dead as well as viable microorganisms contribute to the virulence of a biofilm and should not be ignored.[21] Concerning the reduction in bacterial counts following Er:YAG application, Eick et al. hypothesized that this wavelength (2,940 nm) is rather ablative than bactericidal. Our results support their hypothesis, by showing that all parameter combinations of the laser reduced the biofilm biomass, without affecting the viable bacterial percentage (Figs. 4 through 6). Another study by Hakki et al., published in 2017, also showed similar results to the current study, with superior efficacy using the Er:YAG laser compared with hand curets using failed implants and scanning electron microscope analysis.[22]

Our results support previous studies which evaluated antibacterial efficiency of Er:YAG laser on titanium coated surfaces using different setting combinations. In their review, Mellado-Valero et al.[23] evaluated the available information in the literature on the efficacy of different treatments for decontaminating implant surfaces. The authors stated that some wavelengths, including the 2,940 nm Er:YAG, achieve complete or almost complete elimination of bacteria from titanium surfaces, provided they are used within the appropriate parameters for each surface type. Kreisler et al.[24] showed in their in vitro study that whether with high (120 mJ) or low (60 mJ) energy densities, the Er:YAG laser has high bactericidal potential on common implant surfaces. Tosun et al.[11] found an excellent bactericidal effect of the Er:YAG laser, but in their study, its efficacy was dependent on the frequency and total exposure time of the target to the beam. The results of the current study also show that following Er:YAG treatment, the percentage of viable bacteria mildly increases; while this may contradicts the above publications—it does not. This viability results examines the residual bacteria on the titanium surfaces, and the observed differences do not reflect clinical significant impact.

No clear recommendations could be pulled out from the literature. Each review article on lasers stated as a final note that due to the great heterogeneity of studies, which have used empirical parameters, methods, and implant surfaces, it was impossible to establish a single protocol for optimal implant decontamination. Therefore, the current study tried to address at least one confusing issue, parameter combinations of energy, frequency, and distance from target.

The numerous parameter combinations that were used in our study did not show detectable difference of bactericidal effectiveness. The highest power setting that we used was 2.25W (50 mJ x 45 Hz) and the lowest power was 0.9W (20 mJ x 45 Hz). All parameters demonstrated comparable anti-bacterial effect. The parameter combinations that were chosen by us were similar to those used by Taniguchi et al.[10] who evaluated the thermal damage to implant surfaces subsequent to laser use. They found no significant microstructural and thermal changes following treatment. Stubinger et al.[25] found that distinct alterations with power settings beyond 300 mJ/10 Hz (3W) on SLA surface and 500 mJ/10 Hz on polished surface occurred and warned not to exceed these parameters when Er:YAG is used on those specific surfaces. This corroborates with most available data that showed that with low energies, effective anti-bacterial results are achieved. Kreisler et al. concluded that even at low energy densities, the Er:YAG laser has a high bactericidal potential on common implant surfaces. Hauser-Gerspach et al.[26] who used two laser settings (100 mJ, 10 Hz, 10 seconds and 500 mJ, 10 Hz, 10 seconds) claimed that for maximal decontamination, a higher laser dose was required but alterations of the surface should be expected as opposed to lower doses. As mentioned earlier, these authors commented that the available data for the antibacterial efficacy of Er:YAG laser illustrates enormous heterogeneity of the studies which makes it impossible to draw any conclusions. Another study[27] reported that using Er:YAG laser with power that exceeds 2W causes surface changes on titanium disks. Most studies that used Er:YAG used relatively low energies which were around 1W power and found them efficient.[10,28] Tosun et al.[11] who used similar energy power to ours (0.9W) eliminated 99% to 100% of the bacteria from titanium surfaces. However, they claimed that the impact of frequency and total exposure time on the antibacterial effect was considerable, which was not the case in the present study.

Other than the laser, the conventional cleaning protocols that were used in the present study showed significant residual biofilm on the titanium surfaces following their application. Of interest was the nylon brush results, which show microbial scatter over the titanium surface which extend biofilm distribution. Compared with the above modalities, Er:YAG treatment was demonstrate to be the most effective treatment, leading to clear titanium surfaces with scares of non-vital microbial residues. Furthermore, the present study showed that the Er:YAG effect on the biofilm is related to its light emission and water irrigation properties.

From the present and previous in vitro studies, we can conclude that Er:YAG laser exhibited anti-biofilm properties. However, Er:YAG laser should be tested in vivo to assess their decontamination efficacy on implants and their added value in overcoming the structural complexity of the screw form. Screw shape implants tend to accumulate bacteria in inaccessible grooves, where conventional methods are unable to reach. The implant‘s threads create “blank spots” which are overlooked by the mechanical treatment as with all lasers whose tip cannot access the

infected target directly. On the other hand, Hakki et al., examined various cleaning methods on failed implants, and found similar results to the current study, with better cleaning ability of laser than conventional cleaning methods (excluding air abrasion).[22] Furthermore, the effect of the laser on the titanium surfaces after treatment should be addressed in future studies.

Due to the access limitations of the various instruments, the effect of the laser tip's distance from the target was examined. It is known that in order for the Er:YAG laser beam to reach a target, its tip can be applied in a non-contact mode. This issue is of great advantage for the laser in treating complex anatomical surfaces or sites, and therefore, the effect of the "tip to target" distance was examined.

In conclusion, the present finding demonstrated the possible effectiveness of Er:YAG laser. The demonstration that all three "tip to target" distances (1 to 5 mm) were equally effective—makes it possible for the clinician to avoid the aforementioned anatomical and structural obstacles, and affect the target from several distances in an effective way. However, this hypothesis should be tested in human controlled studies.

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AUTHOR CONTRIBUTIONS

All authors have made substantial contributions to conception and design of the study. David Polak, Mary Alias, and Sharon Shani-Kdoshim have been involved in data collection and data analysis. David Polak, Lior Shapira, and Ayala Stabholz have been involved in data interpretation, drafting the manuscript, and revising it critically and have given final approval of the version to be published.

REFERENCES

1. Alcoforado GA, Rams TE, Feik D, Slots J. Microbial aspects of failing osseointegrated dental implants in humans. *J Parodontol [Internet]*. 1991;10(1):11-18. http://www.ncbi.nlm.nih.gov/pubmed/1906537
2. Becker W, Becker BE, Newman MG, Nyman S. Clinical and microbiologic findings that may contribute to dental implant failure. *Int J Oral Maxillofac Implants [Internet]*. 1990;5(1):31-38. http://www.ncbi.nlm.nih.gov/pubmed/2391137
3. Mombelli A, Buser D, Lang NP. Colonization of osseointegrated titanium implants in edentulous patients. Early results. *Oral Microbiol Immunol [Internet]*. 1988;3(3):113-120. http://www.ncbi.nlm.nih.gov/pubmed/3269993
4. Mombelli A, Lang NP. Microbial aspects of implant dentistry. *Periodontol 2000 [Internet]*. 1994;4:74-80. http://www.ncbi.nlm.nih.gov/pubmed/9673195
5. Aoki A, Sasaki KM, Watanabe H, Ishikawa I. Lasers in nonsurgical periodontal therapy. *Periodontol 2000 [Internet]*. 2004;36(1):59-97. http://www.ncbi.nlm.nih.gov/pubmed/15330944
6. Aoki A, Ando Y, Watanabe H, Ishikawa I. In vitro studies on laser scaling of subgingival calculus with an erbium:YAG laser. *J Periodontol [Internet]*. 1994;65(12):1097-1106. http://doi.wiley.com/10.1902/jop.1994.65.12.1097
7. Ishikawa I, Aoki A, Takasaki AA, Mizutani K, Sasaki KM, Izumi Y. Application of lasers in periodontics: true innovation or myth?. *Periodontol 2000 [Internet]*. 2009;50(1):90-126. http://www.ncbi.nlm.nih.gov/pubmed/19388956
8. Schwarz F, Aoki A, Sculean A, Becker J. The impact of laser application on periodontal and peri-implant wound healing. *Periodontol 2000 [Internet]*. 2009;51(1):79-108. http://doi.wiley.com/10.1111/j.1600-0757.2009.00301.x
9. Schwarz F, Aoki A, Becker J, Sculean A. Laser application in non-surgical periodontal therapy: a systematic review. *J Clin Periodontol [Internet]*. 2008;35(8):29-44. http://www.ncbi.nlm.nih.gov/pubmed/18724840
10. Taniguchi Y, Aoki A, Mizutani K, et al. Optimal Er:YAG laser irradiation parameters for debridement of microstructured fixture surfaces of titanium dental implants. *Lasers Med Sci [Internet]*. 2013;28(4):1057-1068. http://www.ncbi.nlm.nih.gov/pubmed/22886137
11. Tosun E, Tasar F, Strauss R, Kıvanc DG, Ungor C. Comparative evaluation of antimicrobial effects of Er:YAG, Diode, and CO2 lasers on titanium discs: an experimental study. *J Oral Maxillofac Surg [Internet]*. 2012;70(5):1064-1069. http://www.ncbi.nlm.nih.gov/pubmed/22285338
12. Hope CK, Wilson M. Biofilm structure and cell vitality in a laboratory model of subgingival plaque. *J Microbiol Methods [Internet]*. 2006;66(3):390-398. https://linkinghub.elsevier.com/retrieve/pii/S0167701206000042
13. Kolenbrander PE, Andersen RN. Inhibition of coaggregation between *Fusobacterium nucleatum* and *Porphyromonas* (*Bacteroides*) gingivalis by lactose and related sugars. *Infect Immun [Internet]*. 1989;57(10):3204-3209. http://www.ncbi.nlm.nih.gov/pubmed/2777379
14. Genco CA, Cutler CW, Kapczynski D, Maloney K, Arnold RR. A novel mouse model to study the virulence of and host response to *Porphyromonas* (*Bacteroides*) gingivalis. *Infect Immun [Internet]*. 1991;59(4):1255-1263. http://www.ncbi.nlm.nih.gov/pubmed/2004807
15. Miller CH, Palenik CJ, Stamper KE. Factors affecting the aggregation of *Actinomyces naeslundii* during growth and in washed cell suspensions. *Infect Immun [Internet]*. 1978;21(3):1003-1009. http://www.ncbi.nlm.nih.gov/pubmed/30700
16. Herzberg MC, Erickson PR, Kane PK, Clawson DJ, Clawson CC, Hoff FA. Platelet-interactive products of *Streptococcus sanguis* protoplasts. *Infect Immun [Internet]*. 1990;58(12):4117-4125. http://www.ncbi.nlm.nih.gov/pubmed/2254032
17. Shany-Kdoshim S, Polak D, Houri-Haddad Y, Feuerstein O. Killing mechanism of bacteria within multi-species biofilm by blue light. *J Oral Microbiol [Internet]*. 2019;11(1):1628577. http://www.ncbi.nlm.nih.gov/pubmed/31275529
18. Esposito M, Grusovin MG, Worthington HV. Treatment of peri-implantitis: what interventions are effective? A Cochrane systematic review. *Eur J Oral Implantol [Internet]*. 2012;5Suppl(S21):41. http://www.ncbi.nlm.nih.gov/pubmed/22834392

19. Polak D, Maayan E, Chackartchi T. The impact of implant design, defect size, and type of superstructure on the accessibility of nonsurgical and surgical approaches for the treatment of peri-implantitis. *Int J Oral Maxillofac Implants [Internet]*. 2017;32(2):256-362. http://www.ncbi.nlm.nih.gov/pubmed/27643587
20. Eick S, Meier I, Spoerlé F. Vitro-Activity of Er:YAG laser in comparison with other treatment modalities on biofilm ablation from implant and tooth surfaces. In: Leblebicioglu B, ed. *PLoS One [Internet]*. 2017:e0171086. http://www.ncbi.nlm.nih.gov/pubmed/28125700
21. Jun H-K, Jung Y-J, Choi B-K. *Treponema denticola*, *Porphyromonas gingivalis*, and *Tannerella forsythia* induce cell death and release of endogenous danger signals. *Arch Oral Biol [Internet]*. 2017;73:72-78. https://linkinghub.elsevier.com/retrieve/pii/S0003996916302709
22. Hakki SS, Tatar G, Dundar N, Demiralp B. The effect of different cleaning methods on the surface and temperature of failed titanium implants: an in vitro study. *Lasers Med Sci [Internet]*. 2017;32(3):563-571. https://pubmed.ncbi.nlm.nih.gov/28160204/
23. Mellado-Valero A, Buitrago-Vera P, Sola-Ruiz M, Ferrer-Garcia J. Decontamination of dental implant surface in peri-implantitis treatment: a literature review. *Med Oral Patol Oral y Cir Bucal [Internet]*. 2013;18(6):e869-76. http://www.ncbi.nlm.nih.gov/pubmed/23986023
24. Kreisler M, Kohnen W, Marinello C, et al. Bactericidal effect of the Er:YAG laser on dental implant surfaces: an in vitro study. *J Periodontol [Internet]*. 2002;73(11):1292-1298. http://doi.wiley.com/10.1902/jop.2002.73.11.1292
25. Stubinger S, Etter C, Miskiewicz M, et al. Surface alterations of polished and sandblasted and acid-etched titanium implants after Er:YAG, carbon dioxide, and diode laser irradiation. *Int J Oral Maxillofac Implants [Internet]*. 2019;25(1):104-111. http://www.ncbi.nlm.nih.gov/pubmed/20209192
26. Hauser-Gerspach I, Mauth C, Waltimo T, Meyer J, Stübinger S. Effects of Er:YAG laser on bacteria associated with titanium surfaces and cellular response in vitro. *Lasers Med Sci [Internet]*. 2014;29(4):1329-1337. http://www.ncbi.nlm.nih.gov/pubmed/23508281
27. Park J-H, Heo S-J, Koak J-Y, Kim S-K, Han C-H, Lee J-H. Effects of laser irradiation on machined and anodized titanium disks. *Int J Oral Maxillofac Implants [Internet]*;27(2):265-272. http://www.ncbi.nlm.nih.gov/pubmed/22442763
28. Schwarz F, Sculean A, Rothamel D, Schwenzer K, Georg T, Becker J. Clinical evaluation of an Er:YAG laser for nonsurgical treatment of peri-implantitis: a pilot study. *Clin Oral Implants Res [Internet]*. 2005;16(1):44-52. http://doi.wiley.com/10.1111/j.1600-0501.2004.01051.x

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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