Root canals decontamination by coherent photons initiated photoacustic streaming (PIPS) of irrigants: an ex-vivo study

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Abstract. The aim of this *ex vivo* study was to assess the antibacterial effectiveness of coherent photon initiated photoacoustic streaming (PIPS) of irrigants using an Er:YAG laser equipped with a newly designed, stripped and tapered, tip in extracted teeth with infected root canals. One hundred-forty-eight single-rooted extracted teeth were prepared using a rotary abrasive instrument providing a root channel with a suitable size. The samples were sterilized and all teeth except ten (negative control group) were inoculated with *Enterococcus faecalis* and incubated in a CO_2 chamber at 37°C for 15 days in Eppendorff tubes filled with trypticase soy broth medium changed every 2 days. Infected teeth were then randomly divided into 4 test groups (n=32 for each): pulsed erbium:YAG laser at non-ablative settings for 30 seconds with sterile bi-distilled water (Group A) or 5% sodium hypochlorite (NaOCl) (Group B); without laser activated sterile bi-distilled water irrigation for 30 seconds (Group C) or 5% NaOCl irrigation for 30 seconds (Group D); the positive control group received no treatment in infected teeth (n=10). Colony-forming units (CFUs) were counted from bacteriologic samples taken before (S1) and after treatment (S2). Data were analyzed by Kruskal-Wallis and post hoc Dunn's multiple comparison tests. CFU counts were significantly lower in groups B and D than in group C ($P<0.001$). Moreover, there was a significant difference between Group A and C ($P<0.001$). Group B showed the highest CFU reduction, which was significantly greater than that evident in groups A or C ($P<0.001$). There were no statistically significant differences between group B and D ($P>0.05$). None of the four groups predictably generated negative samples. Under the conditions of this *ex vivo* study, statistically significant difference wasn't found in planktonic bacteria reduction between the laser and NaOCl or NaOCl alone groups.

1. Introduction

The first laser specifically designed for dentistry was marketed in 1972. Less than ten percent of dentists world wide own laser, but there are many indications for use for oral procedures [1]. All dental lasers emit either a visible light wavelength or an invisible infrared light wavelength in the portion of that no ionizing spectrum called thermal radiation.

Since human dental tissues are composed of a combination of compounds, the clinician must choose the best laser for each treatment.For soft tissue treatments, the practitioner can use any available wavelength, because all dental lasers absorb one or more of the soft tissue components. For

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hard tissue, however, the Erbium lasers with very short pulse durations easily ablate layers of calcified tissue with minimal thermal effects [2].

The Erbium lasers are absorbed only in a few to several microns of the target tissue's surface, whereas diode lasers can reach a few millimetres deeper into the soft tissues [2].

Dental tissue interaction with laser energy is affected by several factors: wavelength, beam diameter, focused or defocused distance, pulse energy, and providing tissue cooling. The correct combination of all of those parameters should ensure an efficient and beneficial outcome [3]. The currently used dental lasers and their tissue interaction are depicted on the absorption coefficient vs. wavelength of the electromagnetic wave in biological tissues, as reported in Figure 1.

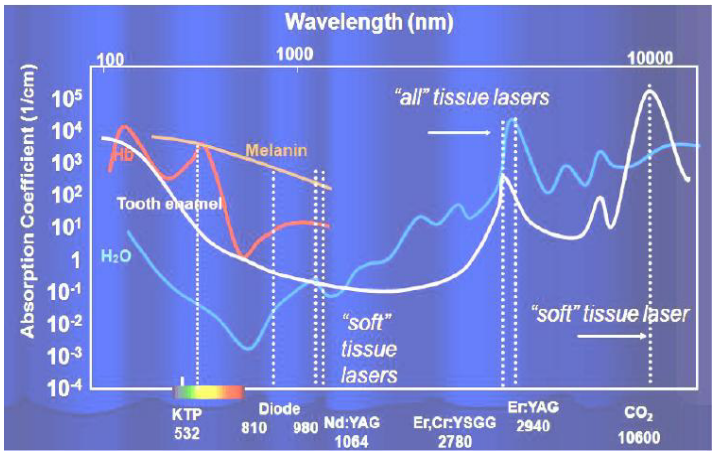

Figure 1. Absorption coefficients of the principal dental tissue components as a function of the laser wavelength.

Recently, the use of different lasers (diode or Er:YAG laser) was proposed in endodontics that is the branch of dentistry for root canal therapy. Microorganisms are the major causative factor associated with endodontic treatment failure. Numerous endodontic protocols use irrigant solutions and mechanical preparation to clean and decontaminate the root canal system [4, 5]. Sodium hypochlorite (NaOCl) has been the most commonly used irrigation solution in endodontics since the early 1900s. It is used alone or in combination with a chelating solution to reduce bacteria and eliminate smear layer. However, studies have shown that the irrigants have a limited ability to effectively reach all internal aspects of seemingly complicated root canal architecture. Different agitation techniques have been proposed to improve the efficacy of irrigation solutions, including agitation with hand files, plastic instruments, sonic, ultrasonic and, more recently, laser devices. Lasers have the ability to clean and effectively disinfect root canals, including elimination of highly resistant species such as *Enterococcus faecalis* [6, 7]. Several authors have investigated different lasers for use during root canal therapy. Currently, visible (532nm), invisible infrared (from 803 to 1064nm) and invisible medium infrared (2780 and 2940nm) wavelengths are commonly used in endodontics. The Er:YAG laser wavelength (2940nm) has the highest absorption in water and high affinity to hydroxyapatite, which makes it suitable for use in root canal therapy. George *et al.* [3] reported the first *in vitro* study to examine the capacity of lasers to activate irrigants inside root canal systems to increase its action on smear layers.

Sahar-Helft *et al.* reported that a combined regimen of a low concentration of chlorhexidine with laser irradiation could be a potential means of inhibiting *Enterococcus faecalis* growth.

Other studies by De Moor *et al.* compared the laser activated irrigation (LAI) to passive ultrasonic irrigation (PUI) on dentine debris. A recent study [8-10] reports the debriding and cleaning efficacy of irrigation enhanced by a new Erbium laser technique that minimizes the morphological thermal effects of infrared radiation. This laser technique uses a photon induced photoacustic streaming (PIPS) of irrigants produced by a newly designed tapered and stripped tip with specific minimally ablative laser setting: low energy (20 mJ); pulse repetition rate (15 Hz) and short pulse duration (50 μs) [11]. The aim of this study was to compare the efficacy of root canal disinfection of *E. faecalis* in extracted teeth

using PIPS [12] (generated by a new Er:YAG laser technique with low energy laser settings plus a new tip for endodontic therapy) or the conventional syringe irrigation technique.

2. Materials and Method

One hundred-forty-eight single-rooted human teeth extracted for periodontal reasons were used in this study. The study protocol was approved by the Ethics Committee of the Catania University. After a conventional access cavities all teeth were instrumented to the anatomical apex by mechanical preparation with Mtwo nickel titanium (NiTi) rotary abrasive instruments (Sweden & Martina, DueCarrare-Pd, Italy) up to the instrument with a tip diameter of 0.25 mm and a taper (final size of the cone) of 60 μm. A coronal reservoir for irrigant placement was created with a Gates Glidden drill #5 placed 5 mm into the canal. Conventional endodontic irrigation with 5% sodium hypochlorite (NaOCl; Niclor 5, OGNA Laboratory, Muggiò, Milan, Italy) and 17% EDTA (OGNA Laboratory, Muggiò, Milan, Italy) was used throughout instrumentation; then all canals were dried using air and sterile paper points. At this point the teeth were placed individually in sterilizer pouches, autoclaved at 134°C for 17 minutes, and stored until use. Ten teeth were not infected and formed the negative control group. All the other 138 teeth were inserted apart in an upright position in the holes of individual sterile Eppendorf vials (Eppendorf Italia, Milan, Italy) filled with trypticase soy broth so that only the apical 2 to 3 mm of the teeth were immersed in the solution. The root canal of each tooth, except for the negative control teeth, were inoculated with 10 μL of a pure culture suspension of *Enterococcus faecalis* (American Type Culture Collection [ATCC] 29212, Oxoid Limited, Basingstoke, Hampshire, United Kingdom - equivalent to Mac Farland 0.5 - $1.5x10^8$ CFU /mL) by sterile syringe and a 30-G irrigation needle (Max-i-Probe; Dentsply Rinn, Elgin, IL, USA) placed as close to anatomical apex as possible. After inoculation, all teeth were incubated at 37°C in a CO_2 chamber to allow growth of *Enterococcus faecalis* for 15 days and each culture medium was replenished every 2 days. Ten out of 138 infected teeth were not treated and formed the positive control group. Then 100 μL of sterile ringer solution were deposited into the root canals and a sterile paper point, with a tip diameter of 0.25 mm and a taper of 20 μm, was placed to working length of each tooth, allowed to saturate and then each placed in a sterile vial containing 2 mL of Trypticase Soy Broth medium (TSB) which was immediately vortexed to collect the initial microbiological samplings (Sampling 1). The 128 experimental teeth were randomly divided into 4 groups (n=32 each). The teeth in group A were treated with bi-distilled sterile water irrigation and laser irradiation for 30 seconds; those in group B were treated with 5% NaOCl irrigation and laser irradiation for 30 seconds; the teeth in groups C were irrigated with only sterile bi-distilled water and those in group D only with 5% NaOCl for 30 seconds. The standardized irrigation protocol involved the use of a 30-G irrigation needle tip introduced as close to anatomical apex as possible without binding and deposition of 3mL of irrigant solution. A final flush was performed with 5mL of sterile 2M sodium thiosulphate solution to inactivate the NaOCl. The laser irradiation protocol was performed by an Er:YAG laser with a wavelength of 2940 nm (Fidelis AT, Fotona, Ljubljana, Slovenia) equipped with a newly designed 12 mm long, 400 μm quartz tip. The tip was tapered and had 4 mm of the polyamide sheath stripped back from its end. Laser operating parameters (using the free-running emission mode) of 20 mJ per pulse, 15 Hz, 50 μs pulse duration, were employed for all of the treatment groups where lasers were used. The co-axial water spray feature of the handpiece was turned off. The tip was placed into the coronal reservoir only and activated for 30s. After canal irrigation had been completed, the remaining intracanal NaOCl was neutralized with 5 mL 2M sodium thiosulphate for 30 s. Then, 10μL of a sterile ringer solution was placed in all root canals and sterile abrasive paper points, with a tip diameter of 0.25 mm and a taper of 20 μm, were inserted to the anatomical apex and left for 60 seconds to soak up the contents of the canals (Sampling 2). The wet paper points were then dropped into sterile Eppendorf tubes (Eppendorf Italia, Milan, Italy) with 1 mL of sterile ringer solution and sonicated for 30 seconds to free the bacteria in solution. Serial dilutions were performed up to a concentration of 10^6. Dilutions from 10^4 to 10^6 were plated on blood agar and incubated at 37°C in a CO_2 chamber for 48 hours. The plates containing more than 30 colonies were counted. The mean number of CFUs for the 10^5 dilution plates

was then calculated, and the results were analyzed by statistical software (MedCalc Software, Mariakerke, Belgium) using Kruskal-Wallis and analysis of variance with Dunn's multiple comparison *post hoc* tests and a P value of <.05 was considered statistically significant.

Fig. 2 shows a photo of the used Er:YAG laser (a), a typical NiTi rotary abrasive instruments to enlarge the root channel (b), a scheme of the procedure of root channel preparation (c), the fibre tip transporting the laser light (d) and, finally, a scheme of the chemically cleaning and debriding of the root canal system using the Er:YAG laser energy at sub-ablative power levels (e).

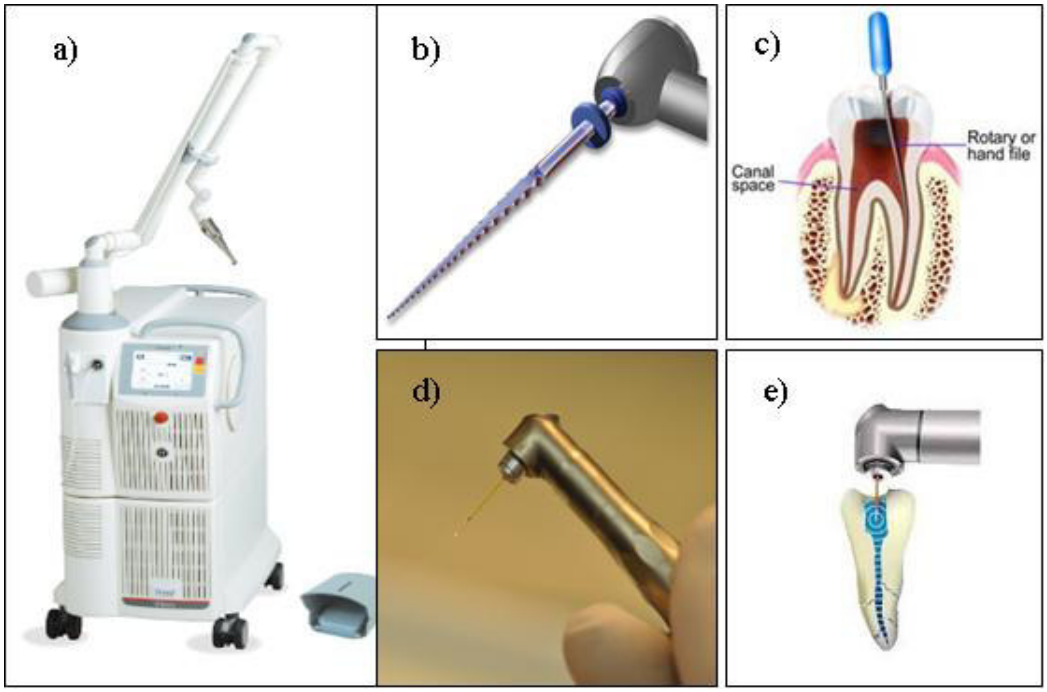

Figure 2. The Er:YAG laser application.

3. Results

Table 1 shows a summary of the results. All of the positive control teeth exhibited high bacterial growth, whereas none of the negative controls showed growth.

After treatment, the number and proportion of negative samples was as follows: 15 for group A (46,87%); 30 for group B (93,75%); 0 for group C (0%) and 25 for group D (78,12%). CFU counts were significantly lower in groups B and D compared to group C ($P<0.001$). Moreover, there was a significant difference between Group A and C ($P<0.001$). Group B (NaOCl irrigation with laser irradiation) showed the greatest percentage bacterial reduction among the groups tested. Moreover, there was a significant reduction of bacteria CFU counts in Group B compared with Groups A or C ($P<0.001$), although no statistically significant differences were found in comparison with group D ($P>0.05$). However, laser-activated irrigation with NaOCl (Group B) was significantly superior to conventional irrigation alone with NaOCl (Group D) in generating negative samples ($P<01$).

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Table 1. Microbiologic mean counts before and after root canal irrigation for the experimental (n=32 each) and control groups (n=10 each).

	Counts before ($x10^6$)		(S1) ($x10^6$)	Counts after ($x10^4$)		(S2) ($x10^4$)	Reduction (%)	Negative culture
	mean	median	range	mean	median	range	median	
Group A	2.84	1.58	7.68 – 0.125	45.9	7.66	0 – 64.5	73.01	15/32
Group B	2.83	1.22	9.15 – 0.247	0.346	0	0 – 5.25	99.80	30/32
Group C	2.58	1.41	13.2– 0.388	139	92.3	18.7– 489	13.04	0/32
Group D	2.92	1.34	12.1– 0.0263	2.54	0	0 – 35.2	97.13	25/32
Ctrl+	2.91	2.72	9.28– 0.797	-	-	-	-	0/10
Ctrl-	0	0	0 - 0	-	-	-	-	10/10

4. Discussion

The present study compared the reduction of *E. faecalis* in straight canals with conventional irrigation to that obtained using photon induced photoacustic streaming (PIPS) of irrigants by an Er:YAG laser with low energy parameters and equipped with newly designed stripped and tapered tips. Four different irrigation protocols were tested: sterile bi-distilled water with or without activation by Er:YAG laser irradiation, or 5% NaOCl irrigation with or without activation by Er:YAG laser irradiation. Many recent publications have focused on the use of the Er:YAG laser. Some have shown positive results, whereas others have reported questionable findings on debris removal or bactericidal effect [7, 10-13]. The results of the present study are suggestive of a positive bactericidal effect of photon - induced photoacustic streaming (PIPS) of irrigants, generated by a pulsed Er:YAG laser equipped with a newly designed quartz tip (400micron, tapered and with 4mm of the polyamide sheath stripped back from its end) but located in the coronal reservoir only. When activated in a limited volume of fluid, the high absorption of the Er:YAG wavelength in water, combined with the high peak power derived from the short pulse duration that was used (50 μs), resulted in a photomechanical phenomenon [11]. Moreover the use of subablative laser settings prevents damage of root canal dentine by a vaporized water mediated explosion [2]. It is suggested that this allows easy access for the photomechanical effects to occur within the root canal, which may assist in cleaning canals of various shapes. Using the tapered and stripped design tips with PIPS, the apex can be reached without the need to negotiate the tip close to the apex. The bactericidal effect of PIPS is non-thermal and does not depend on the canal size as the laser tip is maintained without wall contact in the coronal reservoir [11]. One possible explanation for the effect of laser activation in the present study may be increased NaOCl reaction kinetics [14]. The significant difference between CFU counts of groups A and C ($P<0.001$) seems to show that the PIPS technique is capable of quite well effectively disinfecting the root canals even with sterile water washing by a photomechanical action. However, the significant difference between groups A and B confirms the importance of sodium hypochlorite to obtain a root canals high disinfection (Table I). None of the four groups showed negative samples predictably. Statistically significant differences were not found comparing groups B (NaOCl irrigation + PIPS) and D (NaOCl irrigation only). However, the fact that laser activation generated more negative bacterial samples than conventional irrigation warrants further investigation. In fact, changes in the irrigation protocol could increase the number of bacteria-free cases. Moreover, a longer activation time and possibly changes in the chemical composition of the irrigant, such as adding surface-active agents, might be helpful to enhance deep penetration into dentinal tubules and increase the disinfection of root canals.

5. Conclusions

Under the conditions of this ex vivo study, none of the test groups have removed bacteria from infected root canal systems completely. Water irrigation alone was statistically less effective compared to the other groups in its bacterial load reduction. There were statistically significant differences between bacteria load reduction with or without laser activated bi-distilled water, while there were no statistically significant differences between bacteria load reduction with or without laser activated NaOCl.

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