Bactericidal Effect of Different Laser Systems in the Deep Layers of Dentin

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Background and Objectives: In recent years, various laser systems have gained importance in the field of laser-assisted endodontics, namely the Nd:YAG, the diode, the Er:YAG, and the Er,Cr:YSGG laser. Individual studies have been carried out so far, focusing on the respective wavelength, its specific bactericidal capabilities, and potential usefulness is root-canal disinfection. The present in vitro investigation however, was performed to compare the microbicidal effect of these laser systems under standardized conditions and to draw a conclusion upon their relative effectiveness in the deep layers of dentin.
Study Design/Materials and Methods: In total, 360 slices of root dentin with a thickness of 1 mm were obtained by longitudinal cuts of freshly extracted human premolars. The samples were steam sterilized and subsequently inoculated with a suspension of either *Escherichia coli* or *Enterococcus faecalis*. After the incubation, the samples were randomly assigned to the four different laser systems tested. Each laser group consisted of two different operational settings and a control. The dentinal samples underwent "indirect" laser irradiation through the dentin from the bacteria-free side and were then subjected to a classical quantitative microbiologic evaluation. To assess the temperature increase during the irradiation procedure, additional measurements were carried out using a thermocouple.
Results: Microbiology indicated that all laser systems were capable of significant reductions in both test strains. At an effective output power of 1 W, *E. coli* was reduced by at least three log steps in most of the samples by the tested wavelengths, with the best results for the Er:YAG laser showing complete eradication of *E. coli* in 75% of the samples. *E. faecalis*, a stubborn invader of the root canal, showed minor changes in bacterial count at 1 W. Using the higher setting of 1.5 W, significant reductions of *E. coli* were again observed with all laser systems, where only the diode and the Er:YAG laser were capable of complete eradication of *E. faecalis* to a significant extent. There was no significant relation between the temperature increase and the bactericidal effect.
Conclusions: The present study demonstrates that all the wavelengths investigated are suitable for the disinfection of even the deeper layers of dentin and may prove to constitute valuable tools in state-of-the-art endodontics. Lasers Surg. Med. 35:111–116, 2004.
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INTRODUCTION

The fundamental aim of endodontic therapy is the disinfection of the root canal and its three-dimensional tubular network. Once a bacterial infection of the pulpal tissues has commenced, bacteria also penetrate into the deeper layers of root dentin and propagate a periapical inflammation with subsequent destruction of the adjacent connective tissues [1]. The eradication of persisting bacteria in distant areas of the tubular system is a major challenge in today's treatment regimens and is crucial for the long-term preservation of the endodontically treated tooth.

In the course of the root canal infection, the microenvironment favors the selection of relatively few bacterial types which can survive and proliferate being out of reach of the host's immune response. Rinsing solutions applied during conventional root canal treatment act through direct contact with the bacteria targeted. Due to the insufficient penetration depth of the bactericidal solutions microorganisms in the deeper layers of dentin cannot be affected [2,3]. In addition, bacteria like *E. faecalis* are known to form intra- and extra-radicular biofilms, which makes them even harder to control [4–6]. These facts are often responsible for those cases which are therapy resistant from the beginning or end up as long-term failures after accomplished endodontic treatment.

The introduction of lasers in endodontics has dramatically improved the effectiveness and success rate of root

canal treatment. In general, dental lasers provide greater accessibility of formerly unreachable parts of the tubular network due to their better penetration into dentinal tissues [7–9]. Scientific research was first conducted with the Nd:YAG [10–13] and the diode lasers [14–17] which gained widespread acceptance in the fields of laser-assisted endodontics. For both wavelengths, a high disinfecting capability was reported. At the same time lasers suitable for the preparation of dental hard substances like the Er:YAG and the Er,Cr:YSGG underwent further development resulting in delivery systems also usable for root canal application. Recent investigations indicate that these laser systems exhibit satisfying bactericidal abilities thus constituting relatively new additions to the spectrum of lasers used in endodontics [18–20].

Taking into account these developments the present in vitro investigation was performed to compare the microbicidal effect of four different laser systems, namely the Nd:YAG, the diode, the Er:YAG, and the Er,Cr:YSGG laser, under standardized conditions and to draw a conclusion upon their relative effectiveness. In particular, attention was paid to a study design allowing for the evaluation of laser effectiveness in the deep layers of dentin simulated by indirect irradiation through dentin slices.

MATERIALS AND METHODS

Sample Preparation

Caries-free human premolars were cut into 1 mm thick longitudinal sections using a diamond-coated band saw (Trennschleif System, Exakt, Norderstedt, Germany) under continuous water irrigation. A total of 360 slices measuring 2×6 mm were then cut from the upper and medium third of the dentin adjacent to the root canal. To remove the smear layer resulting from the cutting procedure, the specimens were immersed in an ultrasonic bath with ethylenediaminetetraacetic acid for 4 minutes, followed by three washes in physiological saline solution for a period of 2 minutes each. The samples were stored in physiological saline solution at a temperature of $4°C$ until further use.

Bacterial Inoculation

The samples were steam sterilized (Melatronic 23, Melag, Berlin, Germany) at $134°C$ for 10 minutes to remove all preexisting bacteria. Following this step, they were inoculated with 2 µl of either of the two test strains, *E. coli* (ATCC 25922) or *E. faecalis* (ATCC 29212) on one side by means of a micropipette. Incubation at $37°C$ for 4 hours was carried out to allow the propagation of the bacteria into the dentinal tubules.

Laser Devices

Four different laser systems were applied during the irradiation procedure:

- As an Nd:YAG laser the "Pulse Master 1000" (American Dental Technologies, Texas, USA) emitting at a wavelength of 1,064 nm was used. The variable laser parameters are pulse energy (30–320 mJ) and pulse rate (10–200 Hz) resulting in a power output of 0.2–5 W. The pulse life is 100 microseconds.
- A "LD 15" (Dentek, Graz, Austria) served as a diode laser. Emitting at a wavelength of 810 nm, the laser can be operated in pulsed or CW mode and a repetition rate ranging from 1.5 to 200 Hz, resulting in an output power of 0.5–15 W. Pulse life can be varied between 2 and 32 milliseconds.
- The "Key II", (KaVo, Biberach, Germany), an Er:YAG laser emits at a wavelength of 2,940 nm, with a frequency ranging from 2 to 15 Hz, a pulse energy of 50–400 mJ, and a pulse life of 200 microseconds. This corresponds to a maximum output power of 3.75 W.
- Finally, an Er,Cr:YSGG laser ("Millennium Waterlase", Biolase, San Clemente, CA, USA) emitting at a wavelength of 2,780 nm was used. In this device, pulse energy can be varied between 25 and 300 mJ at a fixed repetition rate of 20 Hz. This results in an output of 0.5–6 W.

Each laser was equipped with a proprietary flexible waveguide and fiber tip with a diameter of 400 µm and was operated in pulsed mode (15 Hz for the diode, Nd:YAG, and Er:YAG; 20 Hz for the Er,Cr:YSGG) without any water spray or air cooling. The lasers were adjusted for an effective average output power of 1 and 1.5 W measured directly on the fiber tip using a wattmeter (Coherent, Inc., Santa Clara, CA, USA) before each irradiation cycle. This procedure ensured stable and standardized irradiation schemes for each sample.

Table 1 illustrates the laser parameters used in each group at an effective output power of 1 and 1.5 W. The power

TABLE 1. Laser Parameters at an Effective Output Power of 1 and 1.5 W

Device	Wavelength (nm)	Pulse rate (Hz)	Pulse energy (mJ) (display)	Output power (W) (display)	Effective output power (W)
Diode	810	15	120	1.8 W	1
			160	2.4 W	1.5
Er:YAG	2,940	15	160	not displayed	1
			220	not displayed	1.5
Er,Cr:YSGG	2,780	20	not displayed	1.5 W	1
			not displayed	2.5 W	1.5
Nd:YAG	1,064	15	80	1.2 W	1
			140	2.1 W	1.5

settings chosen for each irradiation cycle is indicated, depending on the actual transmission of the delivery system.

Laser Irradiation

After incubation, the samples in both groups were divided into nine subgroups, consisting of 20 specimens each. Out these subgroups, eight underwent laser irradiation, the last group of 20 samples served as a control group for each test strain and remained untreated.

The following irradiation procedure was used with all four lasers: The specimens were irradiated from the side opposing the inoculated area in contact mode under constant scanning movement of the optical fibre at an angle of $10°$. One lasing cycle comprised five irradiations of 5 seconds each, with 15 seconds intervals. No water- or air-cooling were used with any device.

Bacteriological Evaluation

Upon irradiation, the specimens were placed into sterile Eppendorf tubes and 100 µl of physiological saline solution were added. Each tube was then vortexed for 1 minute to remove the bacteria from the dentin and the dentinal tubules. The extracted fluid was diluted in log 10 steps. Then, 20 µl of each dilution were applied to culture plates (sheep agar plates, Bio Mérieux, France) and incubated for 24 hours at $37°C$. The colonies were then counted and the total number of bacteria (colony forming units per millilitre of the extraction fluid) was assessed. The lowest detection level of bacteria was 5×10^2 CFU/ml which was decided to represent complete eradication.

Temperature Measurements

To assess the thermal impacts of the different wavelengths and their possible influence on the bactericidal effect, temperature measurements were carried out. For this purpose, five samples were used for each laser and power setting. The dentin slices were mounted on an even thermocouple using a silicon-based heat-conductive compound (Dow Corning 340 Heat Sink Compound, Dow Corning, Midland, Michigan). During the irradiation procedure, which was carried out in the same way as the irradiation of the inoculated samples, the maximum temperature increase (starting from a room temperature of $24°C$) was recorded by the means of a digital thermometer (TMG-1 device, manufactured by the Institute for Applied and Technical Physics, Technical University of Vienna, Vienna, Austria). The average value and the standard deviation of the five measurements per laser/setting were calculated subsequently.

RESULTS

Table 2 shows the results of the bacteriologic test regarding *E. coli* and *E. faecalis*.

Samples are rated in log-steps of the colony counts (CFU/ml), the laser device applied, and the specific radiation powers. Samples with a bacterial count below the detection limit were regarded as eradicated.

The results of the control group of both test strains showed colony counts of about 10^5 CFU/ml demonstrating a decrease of three log steps through the inoculation and incubation process.

Where the laser-induced reduction of *E. coli* is concerned all four systems were capable of eliminating the test strain in the range of one to three log steps at 1 W, when compared to the control group. The Er:YAG yields the best results eradicating *E. coli* in 16 samples with a reduction of at least three log steps.

At 1.5 W bacterial reduction improved for all laser devices. In contrast to the slightly inferior results of the diode laser with 1 W, a massive shift in log steps could be observed with this laser. Again the Er:YAG excelled in the elimination of *E. coli*, achieving complete eradication in all samples.

In general, all four lasers encountered greater difficulties in eliminating the gram positive *E. faecalis*. At 1 W, the diode laser and the Er,Cr:YSGG were capable of removing the germ to an extent of only one log step. The Nd:YAG and the Er:YAG lasers showed a bacterial reduction of around two log-steps.

An increase in effective irradiation power to 1.5 W slightly improved the bactericidal effect of the lasers used. Complete eradication of *E. faecalis* was observed in the minority of the samples evaluated.

TABLE 2. Bacterial Counts of *Escherichia coli* and *Enterococcus faecalis*: for Each Power Setting and Laser Applied the Number of Specimens With the According Range of CFU/ml Is Indicated

	Escherichia coli (CFU/ml)					*Enterococcus faecalis* (CFU/ml)				
	Eradication	10^3	10^4	10^5	10^6	Eradication	10^3	10^4	10^5	10^6
Control				12	8			4	9	7
Nd:YAG: 1 W	7	3	10				3	17		
Nd:YAG: 1.5 W	8	10	2				6	14		
Diode: 1 W	6		8	6				18	2	
Diode: 1.5 W	16	1	3			1	6	12	1	
Er:YAG: 1 W	16	4				2	8		10	
Er:YAG: 1.5 W	20					8		12		
Er.Cr:YSGG: 1 W	4	12	4					17	3	
Er.Cr:YSGG: 1.5 W	7	7	6			1	3	12	4	

TABLE 3. Temperature Measurements. The Averages and Standard Deviations Have Been Calculated From Five Individual Measurements per Laser and Power Setting

Device	1 W	1.5 W
Diode	$4.9 \pm 0.7°C$	$6.6 \pm 0.2°C$
Er:YAG	$6.2 \pm 0.3°C$	$8.5 \pm 0.3°C$
Er,Cr:YSGG	$8.3 \pm 0.7°C$	$8.7 \pm 0.7°C$
Nd:YAG	$5.4 \pm 0.6°C$	$8.2 \pm 0.4°C$

Table 3 presents the results of the temperature measurements. All the measurements were carried out at a room temperature of 24°C, thus they refer to an initial sample temperature of 24°C. For instance, the value 4.9°C stands for a temperature rise to 28.9°C. While the diode laser showed the lowest temperature increases at both power settings, the other devices generated similar thermal effects.

DISCUSSION

The impact of bacterial colonization on pulpal disease and on endodontic treatment failures is a well-known fact. Infections of the root canal system typically have a polymicrobial flora with approximately equal proportions of gram negative and gram positive bacteria [21,22]. These bacteria permeate the three-dimensional tubular network of root dentin and therefore, constitute an essential source for the potential reinfection of an endodontically treated root canal [1]. Consequently the complete removal of the pathogenic bacteria and their toxic byproducts is of crucial importance for the therapy outcome.

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.

Kouchi et al. [2] could demonstrate that bacteria are capable of invading the periluminal dentin up to a depth of 1,100 µm. On the other hand, chemical disinfectants penetrate no more than 130 µm into the dentin as indicated by Berutti et al. [3] 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.

In recent years numerous authors described different laser systems in endodontic treatment as feasible and effective tools for root canal cleansing and disinfection. Traditionally the Nd:YAG laser represents the longest researched device in this field. Hardee et al. [10,11] and Myers and McDaniel [12] were among the first to propose the utilization of this wavelength in endodontics. In an early histological study by Hassan [13] the Nd:YAG laser was found to reduce apical inflammation and accelerate drying of the canal lumen. Experiments like those of Klinke et al. [7] demonstrate that Nd:YAG laser radiation although weakened by penetrating dentin layers has bactericidal effects also in depths of 1,000 µm and above. Studies

by Moritz et al. [23,24] draw similar conclusions. One possible explanation given by Vaarkamp et al. [8] and Odor et al. [9] is the ability of enamel prisms and dentin tubules to act as optical fibers, thus propagating laser light to the dentinal periphery of the root.

Having first succeeded in the fields of ophthalmology and general surgery in the late 80's [25,26] the diode laser was for some time restricted to soft tissue applications. Following an in vitro investigation Moritz et al. [14] introduced this laser system emitting in the near-infrared range to root canal treatment. A follow-up examination of the diode laser in vivo could prove this laser's bactericidal effects under clinical conditions [15]. Comparable results were obtained by other researchers [16,17].

The Er:YAG laser on the other hand is the longest established device in dental hard tissue applications. This laser acts through photoablation since its wavelength correlates closely with the absorption maximum of hydroxyapatite. The mode of action and the device's usefulness in cavity preparation has been demonstrated in various studies by Hibst and Keller [27–31]. For a longer period of time the application of the Er:YAG laser was limited to rigid delivery systems in non-contact mode. The development of superior light conductive materials distinctly broadened the spectrum of this laser's possibilities. In 1997, Hibst et al. proposed the use of this laser in endodontics [18]. Consequently studies by Schoop et al. [19,20] could prove a high bactericidal effect of this wavelength in endodontic procedures.

Another wavelength recently established for the preparation of dental hard tissues is the Er,Cr:YSGG laser. Several articles focused on caries removal and cavity preparation [32–35]. To our knowledge only little is known about this laser as a tool in endodontic treatment especially in regard to its bactericidal effectiveness. While Yamazaki et al. [36] and Kimura et al. [37] describe the morphological changes encountered in irradiated root canal walls no facts are known about the disinfecting abilities.

Until now, not many studies have been conducted directly comparing different laser systems with regard to their bactericidal capabilities. An exception is the work by Moritz et al. [38] which compared the Nd:YAG, the Er:YAG, and the Ho:YAG lasers under in vitro conditions. Freshly extracted and endodontically treated teeth were inoculated and underwent laser irradiation. The Er:YAG laser showed to be the most effective device in terms of bacterial eradication with slightly superior performance than the other laser systems.

The present study has been accomplished in order to directly compare the most promising laser devices in today's endodontics. To facilitate a statement on the bactericidal effects in deeper layers of dentin, the authors followed the procedure described by Moritz et al. in 2000 [24]. Dentin slices were inoculated with the test strains and irradiated from the side opposing the inoculation site.

Considering the actual results, we can draw several conclusions: First, the elimination of gram-negative bacteria like *E. coli* is easier to achieve than that of gram-positive strains with their comparably massive cell-wall-

structure. This effect has been illustrated by Moritz et al. for the Nd:YAG laser [24], but seems to be applicable also for the other devices tested in the present work. Secondly, all tested wavelengths have been able to disinfect the dentin samples to a high extent, again showing the best results for the Er:YAG laser. Finally, the bactericidal potential of Er,Cr:YSGG laser regarding *E. coli* and *E. faecalis* could be illustrated in a controlled in vitro study for the first time.

Using the tested devices under in vivo conditions, one must bear in mind that the "preparation wavelengths" like the Er:YAG and the Er,Cr:YSGG lasers lead to the ablation of dentin at the root canal walls and tend to cause a higher temperature rise [20]. Therefore, exceptional attention has to be paid to irradiation settings which ensure sufficient bactericidal effects on the one hand and avoid the possibility of thermal damage to the surrounding period-ontal tissue on the other. In a study by Schoop et al. [20], endodontically prepared roots when lased with an Er:YAG device at an effective power of 1.3 W exhibit a maximum temperature rise of $4.5°C$. The recommended power setting derived from this investigation was not to exceed 1 W. Therefore, the preparation lasers when applied in root canal treatment without water or air cooling lack the large range of acceptable power settings of the shorter wave-lengths. Yet the present results of the bacteriological evaluation show that even at the lower power setting of 1 W satisfying disinfection can be obtained. Both Erbium devices can be considered valuable tools for today's endodontics.

Finally, the temperature measurements conducted in this study indicate only negligible differences between the various laser systems. No obvious relation can be seen between the differences in temperature change and the distinct variability of the bactericidal effects of each wave-length. The maximum temperature increase of around $8°C$ at 1.5 W does not seem to be of clinical relevance in this context, because of the study design dealing with isolated dentin slices instead of an intact apical environment. As mentioned before, several authors focused on the thermal impacts of the use of lasers in endodontic procedures representing a more appropriate approach to this question from a clinical point of view [14,18,21,38]. Subject to the condition that the temperature increase is the most important factor of bactericidal action, the temperature rise achieved in the present evaluation can be regarded high enough to reduce bacterial viability. However, this fact must be considered in the light of the complex reactions of the cell wall structures on laser irradiation [24] and shall be subject to further investigations.

It can be concluded, that all the wavelengths most commonly used in today's dentistry are also suitable for the disinfection of root canals. The choice of the essential precautions and the correct laser parameters is crucial for a safe and efficient way of therapy.

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