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The Er:YAG Laser in Endodontics: Results of an In Vitro Study

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Background and Objective: Until recently, the main field of Er:YAG laser application was the removal of dental hard substances within the scope of cavity preparation. Nowadays, several new delivery-systems are available, permitting the application of the Er:YAG laser in endodontics. The aim of the present study was to assess the effects of Er:YAG laser irradiation on root canals in vitro.
Study Design/Materials and Methods: For this purpose, 220 extracted human teeth were endodontically processed and subsequently irradiated at different settings using an Er:YAG laser imitating in vivo irradiation procedures. The teeth were then subdivided into three groups and subjected to bacteriological evaluations, scanning electron microscopy, and temperature measurements.
Results: The bacteriological evaluation revealed a decisive bactericidal effect of the Er:YAG laser in the root canal. The bactericidal effect was dependent on the applied output power and specific for the different species of bacteria investigated. Scanning electron microscopy showed discrete removal of dentine from the root canal walls. The temperature rise during irradiation was moderate when standardized power settings were used.
Conclusion: The investigations indicate that the Er:YAG laser is a suitable tool for the elimination of bacteria in root canals under in vitro conditions. Lasers Surg. Med. 30:360–364, 2002. © 2002 Wiley-Liss, Inc.

Key words: bactericidal effect; scanning electron microscopy; temperature measurements

INTRODUCTION

Since the early eighties, laser systems have gained in importance in the field of endodontology. Several authors have studied the impact of these lasers on the root canal and the surrounding dentin.

The CO_2-laser has been used in surgery for quite a long period of time. Zakariasen et al. [1] showed for the first time that this wavelength can also be applied in endodontology with a good bactericidal effect. Moritz et al. [2] achieved a protective coating of dentin tubuli using the CO_2-laser on root canal surfaces. Due to the fact that the emitted long wave infrared radiation (10,600 nm) can be transmitted into the root canal exclusively by the use of a rigid hollow wave guide, the canal lumen must be prepared generously and the laser can be used only in straight root canals.

In vitro studies by Pini et al. [3] and Frentzen et al. [4] focused on the application of the XeCl-excimer-laser, which emits ultraviolet radiation at 308 nm. The low wavelength leads to both a satisfying removal of hard tissues and a bactericidal effect with only limited thermal side effects. The demands on technical resources are tremendous and therefore the utilization of the XeCl-excimer-laser is primarily restricted to basic research.

The most widely used laser in endodontics, the Nd:YAG laser, emits at 1,064 nm. Due to the wavelength in the near infrared range flexible conductors can be used for the application in narrow and bent root canals. This laser yields a bactericidal effect not only on root canal surfaces but also in the deeper layers of dentin. Several studies by Hardee et al. [5], White et al. [6], Rooney et al. [7], Gutknecht et al. [8], and Moritz et al. [9] prove the high bactericidal effect of the Nd:YAG laser.

The diode laser is comparable to the Nd:YAG laser in terms of effectiveness. It emits at a wavelength of 810 nm and possesses comparable bactericidal capabilities as shown by Moritz et al. [10].

For the removal of dental hard tissue the Er:YAG laser provides the most suitable wavelength. Emitting at

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2,940 nm this laser acts through photoablation since its wavelength correlates closely with the absorption maximum of hydroxyapatite. When irradiated, water contained in the dental hard tissue evaporates instantaneously and thereby ablates the surrounding tissue with only minimal thermal side effects. This has been demonstrated in various studies by Hibst and Keller [11–15].

Previously 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. Even teeth with narrow or bent root canals can easily be treated. Hibst et al. [16] described the use of the Er:YAG laser in endodontics.

The present study examines the bactericidal, morphological, and thermal effects of the Er:YAG laser when used as an addition in root canal treatment. In order to evaluate the antimicrobial effect of the laser, bacteriological in vitro experiments with six different species were performed. Morphological alterations on dentinal surfaces were recorded by use of a scanning electron microscope (SEM) and the thermal effects caused by laser irradiation were measured by an infrared camera on the root surface.

MATERIALS AND METHODS

For all in vitro experiments an Er:YAG laser (KaVo Key II, KaVo Biberach, Germany) was used. This laser emits a pulsed infrared radiation at a wavelength of 2,940 nm, with a peak radiation energy of 400 mJ and a maximum repetition rate of 15 pps. Light guidance was performed by a flexible optical fiber and a handpiece specially developed for endodontics and surgical applications. The handpiece provides exchangeable plane fiber tips with a diameter of 400 μm, which can be inserted into the endodontically prepared root canal.

The average laser power emitted at the fiber tip was measured by a wattmeter (FieldMaster, Coherent, Inc., Auburn, CA) before each irradiation to ensure stable and standardized power outputs.

First 220 extracted human teeth with one root were endodontically prepared. The teeth were stored in physiologic saline solution after the extraction. Subsequently trepanation and orthograde enlargement of the root canal to ISO 70 was performed. The prepared teeth were assigned to the three different experimental groups and treated accordingly.

Bacteriologic Tests

The teeth used for these examinations, were autoclaved at 134°C for 5 minutes, to eradicate the preexistent bacterial flora. Each tooth was inoculated with a single strain of diverse aerobic and anaerobic bacteria. Clinical isolates of *Enterococcus faecalis*, *Prevotella buccae*, *Peptostreptococcus micros*, and *Porphyromonas assacharolyticus* were used in addition to the international reference strains *Escherichia coli* ATCC25922 and *Bacteroides fragilis* ATCC 25825.

To inoculate the teeth 10 μl of a bacterial suspension with a bacterial count of approximately 10^8 CFU/ml was filled into the root canal. To get a bacterial suspension with approximately 10^8 CFU/ml 5 ml of a Müller–Hinton broth were inoculated with *E. coli* or *E. faecalis* and incubated overnight.

For the anaerobic bacterial suspension a Sabouroud-Glucose broth was used. After inoculation with anaerobic bacteria the broth was incubated up to 72 hours.

The inoculated teeth were sealed with wax, and then placed into sterile microcentifuge tubes containing 100 μl of a physiological saline solution. After an incubation period of 4 hours at 35°C under aerobic and anaerobic conditions, the teeth were taken out of the Eppendorf tubes and the wax seal was removed.

Now, laser irradiation was performed in the root canal. For each strain the following experimental proceeding was applied: Thirty-three samples were prepared. Three groups of ten samples each underwent laser treatment at a setting of 80, 180, and 250 mJ (as indicated at the display of the laser unit) and with an actual power output of 0.5, 1, and 1.3 W, measured directly at the end of the fiber tip. The pulse rate was the same for all groups (15 Hz). Each sample was treated with one lasing cycle, which comprised of five irradiations of 5 seconds duration with 20 seconds break in between. For irradiation the optical tip was inserted as far as the apex. Then the laser was activated and the root canal was *continuously* radiated from apical to coronal in slow, circling movements. By means of this procedure the irradiation of the entire root canal could be ensured.

Additionally, in each group one sample was excluded from laser irradiation and thus served as a control group. Immediately after the laser treatment the root canal was rinsed with 1 ml of a physiological saline solution, and the eluate was collected in a microcentrifuge tube.

Finally, the bacterial count was determined. The extracted fluid was diluted in log 10 steps. Twenty microliters of the dilution were applied to culture plates (Columbia Agar plates with 5% sheep blood, BioMerieux, France) and the aerobic and anaerobic bacteria were incubated for 20 and 72 hours, respectively. The colonies were counted, and the total number of bacteria per milliliter of the extracted fluid was assessed. The lowest detection-level of bacteria was 5×10^2 CFU/ml, which was decided to represent complete eradication.

Scanning Electron Microscope

For this experiment 12 extracted teeth with one root were used. Irradiation was performed in steps of 0.5, 1, and 1.3 W, corresponding to 120, 180, and 250 mJ at 15 Hz, for 5×5 seconds. Afterwards the teeth were cut longitudinally to expose the root canal and examinations by SEM with 150 × and 2,000 × enlargement were performed. A JEOL 330A (Jeol, Inc., Tokyo, Japan) SEM was utilized. The specimens underwent drying and gold sputtering prior to the SEM procedure.

Temperature Measurements

In order to measure the temperature rise during laser radiation measurements were performed on ten extracted

human teeth with one root by using an infrared camera (Avio TVS, Cincinnati Electronics, Mason, Ohio). These measurements were done at 0.5, 1, and 1.3 W, 15 Hz and for 5×5 seconds. First the root canals were dried with conventional paper tips (ROEKO, Langenau, Germany) imitating in vivo endodontic procedures and then the teeth were attached to silicon-based fixtures at a distance of 30 cm to the camera. A series of three measurements at the different power settings was carried out with each tooth. Laser irradiation was done five times for 5 seconds with breaks of 20 seconds in-between analogous to the procedures mentioned above. During this process, the thermographic images were videotaped and stored for the final evaluation. The highest value observed in each measurement was used for the calculation of the mean temperature rise for the three power settings.

RESULTS

Bacteriologic Tests

Table 1 shows the results of the bacteriologic tests. Samples are rated by colony count (CFU/ml) and radiation power (log). Samples with a bacterial count below the detection limit were regarded as eradicated.

The results of the control samples showed colony counts of about 10^6 CFU/ml, demonstrating a decrease of 2 log steps through the inoculation and incubation process. Laser radiation at 1 W yielded a bacterial reduction by 4 log steps, corresponding to 99.99% reduction in every species but *E. faecalis*. However, at an output power of 0.5 W only Prevotella buccae could be eradicated completely. *E. faecalis* could not be eradiacted even when using 1.3 W. Three samples inoculated with this germ still revealed a bacterial concentration of 10^3 CFU/ml at this power setting.

Scanning Electron Microscope

At low magnification, Er:YAG laser treated root canals showed the typical surface structure resulting from photoablation. Figure 1 shows the root canal wall at 150-fold magnification after irradiation with 1 W. At the right margin the adjacent periluminal dentin can be seen.

Figure 2 gives a detailed view of the root canal wall at 2,000-fold magnification after radiation with 1 W. The exposed dentinal tubules are clearly discernible, since the smear layer resulting from manual preparation had been removed by laser irradiation.

When power was increased to 1.3 W, melted and recrystallized ablation products can be seen in some places (Fig. 3, 2,000-fold magnification).

Temperature Measurements

Temperature measurements showed an average rise in temperature at the root surface of $2.6 \pm 0.32^{\circ}$C, at a laser setting of 0.5 W, 15 Hz. When the power setting was increased to 1 W, a mean temperature rise of $3.1 \pm 0.28^{\circ}$C was observed. The highest rise in temperature was $4.5 \pm 0.38^{\circ}$C during the irradiation process with 1.3 W as indicated in Figure 4.

TABLE 1. Bacterial Count: For Each Power Setting the Number of Specimens With the According Range of CFU/ml is Indicated

CFU/ml	0.5 W	1 W	1.3 W	Control
Escherichia coli				
Eradication	7	10	10	
10^3	2			
10^4	1			
10^5				
10^6				3
Bacteroides fragilis				
Eradication	7	10	10	
10^3	2			
10^4	1			
10^5				
10^6				3
Prevotella buccae				
Eradication	10	10	10	
10^3				
10^4				
10^5				
10^6				3
Peptostreptococcus micros				
Eradication	8	10	10	
10^3	2			
10^4				
10^5				1
10^6				2
Porphyromonas asacharolyticus				
Eradication	9	10	10	
10^3				
10^4	1			
10^5				2
10^6				1
Enterococcus faecalis				
Eradication	2	6	6	
10^3	8	4	4	
10^4				
10^5				
10^6				3

DISCUSSION

Successful endodontology relies to a great extent on complete cleaning of the root canal. Infected dentin and pulpal tissue can endanger therapy outcome. Convential root canal treatment aims at the removal of the infected pulp and dentin layers by using mechanical techniques and bactericidal irrigants. Several studies indicate that these techniques are only partly successful. By using lasers better results can be achieved [17–20].

Studies by Kouchi et al. [21] show that bacteria colonize the periluminal dentin up to a depth of 1,100 μm. Chemical desinfectants penetrate only 100 μm into the dentin, as indicated by Berutti et al. [22]. In addition, bent root canals or side-branches can be obstacles in the conventional root canal treatment.

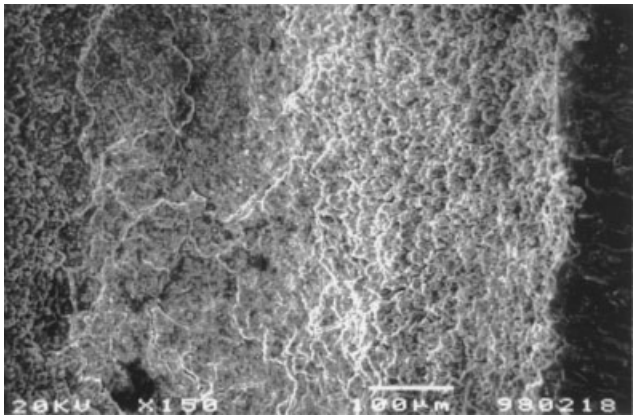

Fig. 1. Scanning electron microscope (SEM) image of the root canal wall after irradiation at 0.5 W, 5×5 seconds. Longitudinal cut through the root, magnification $\times$ 150, 20 kV.

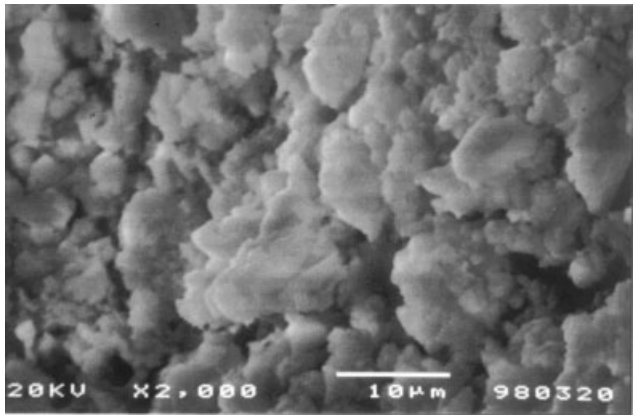

Fig. 3. SEM image of the root canal wall after irradiation at 1.3 W, 5×5 seconds. Longitudinal cut through the root, magnification $\times$ 2000, 20 kV.

Several experiments, like those of Klinke et al. [23] 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. [24] draw similar conclusions. One possible explanation given by Vaarkamp et al. [25] and Odor et al. [26] is the ability of enamel prisms and dentin tubuli to act as optical fibers. Using the Er:YAG laser, Schoop et al. [27] could achieve a comparable bactericidal effect on *E. coli* under in vitro conditions.

In the present study, a conventional endodontic treatment regimen was augmented by the use of the Er:YAG laser. The aim was to evaluate the effectiveness of the 2,940 nm wavelength in endodontology.

Even at the lowest power setting (0.5 W) a distinct reduction in bacterial counts could be observed, although bacterial reduction below 10^2 CFU/ml could not be achieved in all the samples. Specimen irradiated at 1 W did not show bacterial growth at all with the exception of *E. faecalis*. This species could not be eradicated even when exposed to 1.3 W of laser irradiation. Therefore irradiation settings of more than 1 W are not necessary to eradicate most of the endodontic bacterial species.

The complete eradication of *E. faecalis* would require power settings that bear the risk of severe thermal side effects and ultimately damage to the surrounding periodontal tissue. The resistance of *E. faecalis* to laser exposure can be explained by the cell wall structure of the gram-positive bacteria [24]. This germ is also a problem in conventional root canal treatment where it often persists as a monoinfection and represents one of the most stubborn invaders of periluminal dentin [28].

This investigation indicates that under in vitro conditions, the Er:YAG laser shows a similar bactericidal effect compared to other laser systems used in root canal treatment. These systems (Nd:YAG and diode laser) yielded a

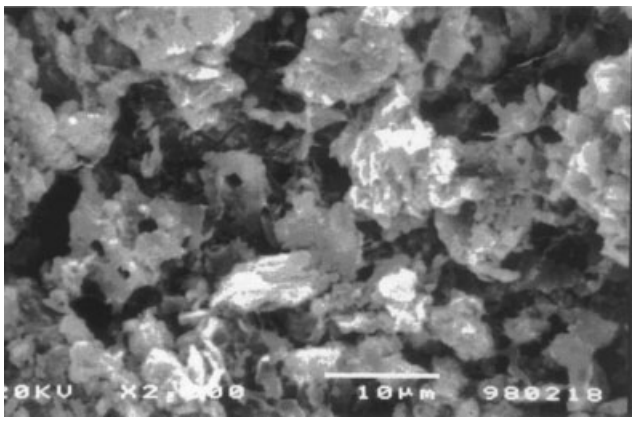

Fig. 2. SEM image of the root canal wall after irradiation at 1 W, 5×5 seconds. Longitudinal cut through the root, magnification $\times$ 2000, 20 kV.

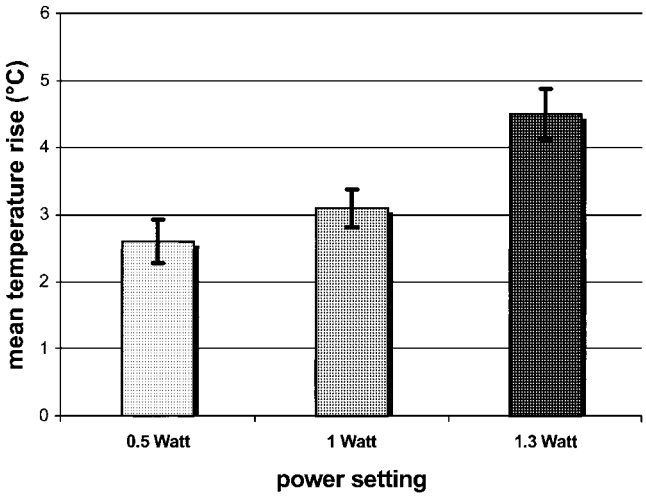

Fig. 4. Mean temperature rise during irradiation at power settings of 0.5, 1, and 1.3 W.

bacterial reduction by three to four log steps in vitro as well as in vivo [8,10].

The Er:YAG laser differs distinctly from other laser systems regarding the effect on the root canal wall, as can be seen in the present SEM investigation. The Er:YAG laser is capable of removing infected dentinal surfaces and the ubiquitous smear layer present after all forms of mechanical root canal preparation. The orifices of the dentinal tubules are exposed facilitating a tight fitting root canal filling, which is indispensable for a successful endodontic treatment.

The temperature changes on the root surface recorded by the infrared camera are within acceptable limits when laser settings do not exceed 1 W at a duration of 5 seconds and the laser fiber is kept in constant motion to avoid unjustifiably high temperatures at the apical periodont.

However, due to the study design, only limited information is available about the bactericidal effects of the Er:YAG laser in the deep layers of dentin. Further examinations, comparable to those conducted by Moritz et al. [24] are necessary. The same holds true for the evaluation of the interactions of laser and bacteria and the mechanisms of action (like thermal, drying, shock waves, and cavitation effects).

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