

# Antibacterial Effect of Er:YAG Laser in the Root Canal

Silvana Jukić<sup>1</sup>  
Darija Vidučić<sup>2</sup>  
Ivana Miletić<sup>1</sup>  
Želimir Božić<sup>3</sup>  
Smiljana Kalenić<sup>4</sup>  
Ivica Anić<sup>1</sup>

<sup>1</sup>Department of Dental Pathology  
School of Dental Medicine  
University of Zagreb  
<sup>2</sup>Department of Microbiology  
School of Dental Medicine  
University of Tokushima  
Tokushima, Japan  
<sup>3</sup>Private Practice Prestranek  
Slovenia  
<sup>4</sup>Department of Microbiology  
School of Dental Medicine  
University of Zagreb

---

## Summary

*The aim of this study was to evaluate the antibacterial effect of Er:YAG laser in the root canal using bacteria of Enterococcus and Staphylococcus species.*

*Eleven single-rooted teeth were instrumented, sterilized and ten of them inoculated with bacterial suspension of concentration 10<sup>6</sup> CFU/ml in brain heart broth. Nine samples were irradiated with Er:YAG laser (220 mJ/40 Hz/10 s) in two cycles with a pause of 15 s for cooling. One sample with bacterial suspension without irradiation was a positive control, and one sterile sample a negative control. After irradiation the samples were placed in vials with broth and the number of CFU/ml was determined by bacterial analysis after one and two weeks. Er:YAG laser did not completely sterilize any canal, but in three cases elimination of Staphylococcus aureus occurred. This indicates the possible better antibacterial effects of erbium laser in the root canal if appropriate energy delivery system could be applied.*

**Key words:** *Er:YAG laser, Staphylococcus aureus i Enterococcus faecalis, antibacterial effect.*

---

Acta Stomat Croat  
2001; 201-204

ORIGINAL SCIENTIFIC  
PAPER  
Received: January 25, 2001

Address for correspondence:

Silvana Jukić  
Department of Dental Pathology  
School of Dental Medicine  
University of Zagreb  
Gundulićeva 5, 10000 Zagreb  
Croatia

---

## Introduction

The persistence of bacteria in the root canal system after endodontic treatment often leads to failure of the treatment (1,2). Mechanical reduction of microorganisms in the root canal could be

achieved by machine or manual instrumentation and irrigation with solutions that also have a chemical action on microorganisms. Most of the solutions used in endodontic practice today show high antibacterial effects. One of the most effective solutions is sodium hypochlorite. On account of its high pH,

sodium hypochlorite dissolves proteins, forming chloramines residues on the remaining peptide fragments, thus not only aiding debridement but also contributing to the antimicrobial action of the free chlorine. Furthermore, it inactivates the sulfhydryl groups of bacterial enzymes by forming hypochlorous acid (3). One of the disadvantages of antibacterial solutions is their limited penetration in dentine tubules, which compromises antibacterial action (4), and the time needed for their efficiency. Penetration, even in small amounts, of sodium hypochlorite in periradicular tissue, has toxic and allergenic effect thus promoting reactive inflammation (5). A concentration of 1.0% NaOCl has been suggested as optimal, balancing toxicity against antimicrobial effectiveness (6,7). Moreover, some gram+ bacteria such as *Streptococcus lactis* and aerococci showed resistance to the NaOCl (8).

Antibacterial effect of laser beam is based on the thermal properties of laser tissue interaction (9). Reduction, or even complete elimination, of bacteria has been achieved by several laser systems (6,9,10). The Erbium laser is a very promising laser system as the emission wavelength of 2.94  $\mu\text{m}$  coincides with the main absorption peak of water, resulting in good absorption in all biologic tissues, including enamel and dentine (11). The Erbium laser is recommended for osteotomy, cyst removal or apicotomy because of excellent bone healing. It is the only one laser approved by FDA (Food and Drug Administration) for cavity preparation (12).

The introduction of lasers in endodontics has been made possible by the development of fiber delivery system (13). On account of great energy loss, few erbium laser units are equipped with this fiber delivery system. Therefore, application of this laser system in endodontic practice is still restricted.

The aim of this study was to investigate the antibacterial effect of Er:YAG laser in root canals infected with *Enterococcus faecalis* and *Staphylococcus aureus*.

## Materials and methods

### Laser device

In this study we used Twilight Dental Laser (Fotona, Slovenia) which is equipped with Er:YAG and Nd:YAG laser subunits.

Er:YAG laser in the pulsed mode at 2.94  $\mu\text{m}$  was used. Frequency ranged from 2-10 Hz, energy from 0.12-5 W and pulse duration from 250-45 s depending on the energy of the pulse. The energy was delivered through the flat-polished end of the 900  $\mu\text{m}$  core diameter fiber.

### Sample preparation

Eleven extracted human single rooted teeth were used for this study. After extraction the teeth were stored in 10 % Formalin solution.

The crowns were removed at the level of the cemento-enamel junction using a high-speed diamond disc with water-cooling system.

Endodontic space was coronary flared with Gates Glidden burs #2 #3 and #4. The canals were shaped using standard step back technique to a size ISO 50 # K-file (Maillefer, Ballaigues, Swiss) at the working length, which was determined 1 mm short of the apical foramen. During the instrumentation, each root canal was irrigated with a 5 ml 2.5% NaOCl throughout. The teeth were then allowed to air-dry overnight and the apical foramina was sealed with coats of clear nail polish. The teeth were placed for sterilization by ethylene oxide gas for 24 h and left undisturbed for 7 days.

### Inoculation

Equal amounts of bacterial suspension of *Staphylococcus aureus* ATCC 29 213 and *Enterococcus faecalis* ATCC 29 212 were cultivated in Brain-Heart broth in a concentration of  $10^6$  CFU/ml. The concentration was previously examined by overnight culture growth on blood agar (bioMérieux, Mercy l'Etoile, France) (Murray et al. 1999).

Immediately before the irradiation a micropipette with sterile needle was used to inoculate the canals with 10  $\mu\text{l}$  of the bacterial suspension in nutrient solution. The negative control was not inoculated.

### Lasing procedure

The root canals were irradiated with the Er:YAG laser in pulse mode (220 mJ/10 Hz/10 s). without moving the laser tip. The irradiation was performed twice with 15-s recovery period for cooling. The duration of the output pulse was 330 s. One specimen served as a negative control and another as a positive control.

During the whole experiment, teeth were kept in plastic bags that were used during the sterilization procedure. In this way we tried to minimize potential infection from the surrounding area. Negative control was also irradiated in contrast to the positive control which was treated with optical fiber without a turned on laser device.

After the laser irradiation the teeth were placed in vials which contained 2 ml of medium.

### **Bacteriological analysis**

The vials were incubated for 15 days at 35°C. After incubation for 24 hours 0.5 ml of BHI was taken from each specimen and one half inoculated in salty brain-heart broth (BBL, Becton Dickinson Microbiology System Cockeysville MD 21030, USA) for selective isolation of *Staphylococcus*; the other half was added to enterococose broth (BBL, Becton Dickinson Microbiology System Cockeysville MD 21030, USA) for selective isolation of *Enterococcus*. These broth samples were incubated for a further 15 days. Vials with teeth and inoculated salty brain-heart broth samples were examined every 24 hours, and if they became turbid they were planted on blood agar. Blood agars were incubated at 35C in the air for 24 hours, and grown colonies were identified by standard methods. Inoculated enterococose broth samples were likewise examined every 24 hours, and in case of darkening they were planted on enterococose agar. Grown colonies were identified using standard methods (14).

### **Results**

The results are shown in Table 1. Er:YAG laser, with the used parameters, did not sterilize any samples, although in three cases there was a significant reduction in *Staphylococcus aureus* in the first and second week

### **Discussion**

*Staphylococcus aureus* and *Enterococcus faecalis* are considered to be normal inhabitants of the infected endodontic space (15). Due to fact that the antibacterial property of lasers is mostly based on

the thermal effect the important characteristic of the bacteria used in laser studies is their thermoresistance. *Enterococcus faecalis*, however, non-sporiferous vegetative bacteria, is resistant to high temperatures (16). *Staphylococcus aureus* is somewhat more heat-susceptible than bacteria *Streptococcus* species (10). This is in accordance with our findings. In three samples selective destruction of bacterial species more susceptible to the heat occurred.

In their study Hardee et al. (6) achieved a reduction of CFU from  $2.3 \times 10^6$  to  $0.6 \times 10^6$ , after 1 min of lasing with 3.5 W. This represents a 98 % reduction in CFU. As a test organism they used *Bacillus Stearothermophilus* which is a heat resistant spore. Although they achieved significant CFU reduction, the authors describe a high elevation of the temperature on the root, which made it difficult to hold the sample in the fingers. These results refer to possible periodontal injuries, which have been confirmed in a study by Bachall et al. (17) conducted on dogs. Irradiation of root canals by 3 W / / 30 s caused ankylosis, cemental resorption and major bone remodeling found in histological analysis 30 days post treatment (18).

Investigations by Moritz et al. (19) with Nd:YAG laser *in vivo*, gave encouraging results in reduction of bacteria present in the root canal. With a repeated laser beam five times for 10 s, with recovery period of 20 sec, 1.5 W and repetition rate of 15 pulses/s they managed to achieve 50 % reduction of bacteria specimens in the first attempt. Calculation gave the highest log-ratio for *Streptococcus* 3.9 and *Staphylococcus* 4.32. However, in these studies material for the bacterial sampling procedure was collected by irrigating the canal with saline solution and inserting a sterile paper point. This method allows bacteria to adhere to the root canal surface and hide in dentinal tubules, and is therefore not present in representative material. This could be confirmed by the low recovery rate of spores, up to 25 %, that Hardee et al. (6) obtained in a control sample by flushing of inoculated canals. In this investigation, the samples were placed in vials with brain-heart broth immediately after the lasing procedure in order to include the hidden and adhered bacteria in the examination sample and avoid dryness of the teeth. In addition, the duration of the experiment

(15 days) made possible the recovery of bacteria expected to occur in clinical conditions.

Although erbium laser did not completely sterilize any root canal, a reduction of *Streptococcus aureus* was noted in three cases. These results can be explained by the fact that the erbium laser used in this study was equipped with a laser tip which is only applicable in the cervical third of the root canals. On the basis of this finding we assume that Er:YAG laser equipped with fiber of smaller diameter and longer, that could be inserted into the root canal, would possibly achieve a higher reduction rate of bacteria. The erbium laser beam ( $\lambda=2.94\mu\text{m}$ ) is highly absorbant in water, which represents the main component of bacterial cells (11).

The significant antibacterial effect of Er:YAG laser with low energetic parameters was demonstrated in an *in vitro* study where the bacteria frequently found in periodontal pockets were exposed to the laser beam. Application of low energy levels ranging from 7.1 to 10.6 J/cm<sup>2</sup> reduced the number of survived *Porphyromonas gingivalis* significantly (18). Also, more recent investigations on the appli-

cation of this laser in the root canal are proving its efficient antibacterial action (19,20).

Although the Er:YAG laser did not significantly reduce the number of bacteria, the fact that in spite of non-appropriate delivery system for the endodontic space the colony number of *Staphylococcus aureus* was reduced in three samples, indicates its possible antibacterial usage in the future. This would be possible by the introduction of an optical fiber that is already used for experimental purposes (13).

### Conclusion

Er:YAG laser beams did not sterilize any sample. Antibacterial non-effectiveness could be explained by the lack of an appropriate energy delivery system for the endodontic space. Nevertheless, complete elimination of *Staphylococcus aureus* in three samples indicates its possible use in endodontics which could be achieved by the introduction of an optical fiber.