ANTIBACTERIAL EFFECTS OF Nd:YAG LASER IN ROOT CANAL SAMPLES: *IN VITRO* STUDY

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SUMMARY – The aim of the study was to investigate the antibacterial effect of Nd:YAG laser in root canal samples using suspension of *Enterococcus faecalis* and *Staphylococcus aureus*. Twenty-two single-rooted teeth were instrumented, sterilized and inoculated with bacterial suspension concentration of 10^6 CFU/ml in brain-heart broth. The samples were irradiated by Nd:YAG laser, one half with power setting at 3.5 W, and the other half with 2.5 W, frequency of 40 Hz, in five cycles of 15-s lasing and 15-s pause for cooling. After irradiation, the samples were placed in vials with broth and the number of CFU/ml was determined after one and two weeks. Laser beams of 3.5 W power setting sterilized only three samples in the first week. In one of them, the bacteria predominated by *Enterococcus faecalis* grew in the second week, whereas pure culture of *Enterococcus faecalis* grew in another sample. Irradiation by Nd:YAG laser with 2.5 W did not sterilize any of the samples. Nd:YAG laser with the parameters used in this study did not significantly reduce the number of bacteria in root canal samples.

Key words: *Root canal therapy – methods; Lasers – therapeutic use; Root canal preparation – instrumentation; Dental pulp cavity – microbiology; Disinfection – methods*

Introduction

The persistence of bacteria in the root canal system after endodontic treatment often leads to failure[1,2]. Mechanical reduction of microorganisms in root canals could be achieved by machine or manual instrumentation and irrigation. Only instrumentation of the root canal is inadequate for its thorough cleaning and disinfection during endodontic treatment. Thus, instrumentation should be combined with chemical treatment for the procedure to be fully successful. Most of the solutions that are currently used in the endodontic practice show high antibacterial effects. One of the most effective solutions is sodium hypochlorite (NaOCl). Sodium hypochlorite dissolves proteins, forming chloramine residues on the remaining peptide fragments, thus not only aiding in 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]. Two of the disadvantages of antibacterial solutions are their limited penetration into dentin tubules, which compromises antibacterial action[4], and the time needed for their efficacy. Penetration, even in small amounts, of sodium hypochlorite in the periradicular tissue has a toxic and allergenic effect, thus promoting reactive inflammation[5]. A concentration of 1.0% NaOCl has been suggested as optimal, balancing toxicity and antimicrobial activity[6]. Moreover, some gram-positive bacteria such as *Streptococcus lactis* and *Aerococcus* showed resistance to NaOCl[7].

In order to achieve better results of endodontic treatment, a great deal of effort has been made to find another approach. The past few years have witnessed a strong emergence of lasers in dentistry. The antibacterial effect of a laser beam is based on thermal properties of the laser tissue interaction[8]. In the study of Zachariasen *et al.*[9], the *in vitro* bactericidal action of CO_2 laser on six different types of bacteria was demonstrated both in teeth and on glass slides.

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The introduction of lasers in endodontics has been made possible by the development of a fiber delivery system. This technology enables laser beam delivery directly into the root canal through optic fiber, and many studies of its effectiveness in canals have been conducted[10]. The advantages of Nd:YAG laser have been shown in many investigations: cleaning and shaping[11], sealing of dentinal walls[12], and sterilization of the root canal[6]. It is well known that when the laser energy is absorbed, the temperature in the tissue rises. The elevation of the temperature in the canal and its transmission to the root surface can cause damage to the surrounding periodontal tissue. The critical temperature for bone injury lies at 47 °C, only 10 °C above human body temperature[12]. Denaturation of serum and tissue proteins occurs at prolonged temperature of 65 °C to 70 °C.

Use of the pulse mode of laser energy, including intervals needed for cooling and water-cooling system, can reduce the temperature on the root surface[13]. The Nd:YAG laser beam, with a wavelength of 1064 nm, is to a great extent absorbed by blood, hemoglobin, melanin pigments and other dark pigmented tissue components, which contribute to its thermal effects. Exogenous dyes such as Indian ink or Nigrosin can be used to enhance absorption in tissues and induce interactions that otherwise might not occur.

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

Materials and Methods

Laser device

In this study, we used an Nd:YAG laser (Twinlight Dental Laser, Fotona, Slovenia). This laser operates at a wavelength of 1064 nm with a pulse duration of 125-160 ms, pulse repetition rate of 10-100 Hz, and energy ranging from 0.5 to 8 W. The laser beam is delivered *via* a 320-nm fiberoptic system. A red diode with a wavelength of 670 nm and energy of 1 mW was used to aim the beam.

Sample preparation

Twenty-two extracted human single-rooted teeth were stored in 10% formalin solution. The crowns were removed at the cemento-enamel junction using a high-speed diamond disk with water cooling system. The canals were shaped using standard step-back technique to a #50 K-file (Maillefer, Ballaigues, Switzerland) at the working length, which was determined by withdrawing the instrument 1 mm short of the apical foramen. For irrigation after each instrument, 5 mL of freshly prepared solution of 2.5% sodium hypochlorite (NaOCl) were delivered. The coronal part of the root canals was flared with #2, #3 and #4 Gates Glidden drills (Maillefer, Ballaigues, Switzerland). The teeth were then allowed to air-dry overnight, and the apical foramen was sealed with coats of clear nail polish. The teeth were sterilized using ethylene oxide gas and left undisturbed for 7 days.

Inoculation and lasing procedure

Equal amounts of bacterial suspension of *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were cultivated in brain-heart broth in a concentration of 10^6 CFU/ml. The concentration was previously examined for overnight culture growth on blood agar (bioMérieux, Mercy l'Etoile, France)[14]. With a sterile needle (Microlance 3, Becton Dickinson, Spain) mounted on a micropipette, a fixed volume (1 ml) of black dye was inserted into all root canals before the addition of the bacterial suspension. Immediately before irradiation, a micropipette with a sterile needle was used to inoculate the canals with 10 ml of the bacterial suspension in nutrient broth. The teeth were randomly assigned to three groups that received different laser treatments.

The samples were treated with the Nd:YAG laser with energy set at 2.5 W and 3.5 W, and frequency of 40 Hz. For each energy level teeth were lased for five 15-s periods with a 15-s recovery period between lasing sessions[15]. The pulse duration was 140 ms. Two teeth served as negative control and were neither inoculated with bacterial suspension nor received laser treatment. Another two teeth served as positive control and received only bacterial inoculation. Before the lasing procedure, optic fiber was inserted in the root canal, approximately 2 mm from the apex. During the treatment, the fiber was slowly moved up and down in the canal with a spiral movement. The lasing with the energy set at 3.5 W produced high elevation of the temperature at the root surface, which made the teeth very hot to touch. Throughout the experiment, the teeth were wrapped in plastic bags that were used during the sterilization procedure. In this way, we tried to minimize potential infection from the surrounding area. After laser irradiation, the teeth were placed in vials, which contained 2 ml of the medium.

Bacteriologic analysis

The vials were incubated at 35 °C for 15 days. This period of time was assumed to allow for possible recovery of bacteria. After 24-hour incubation, 0.5 ml of BHI were

taken from each vial with samples, and one half was inoculated in salty brain-heart broth (BBL, Becton Dickinson Microbiology System, Cockeysville, MD, USA) for selective isolation of *Staphylococcus* spp. The other half was added to enterococosel broth (BBL, Becton Dickinson Microbiology System, Cockeysville, MD, USA) for selective isolation of *Enterococcus* spp.[14] These broth samples were incubated for 15 more days. The vials with teeth and inoculated salty brain-heart broth samples were examined every 24 hours, and if they had become turbid they were plated on blood agar. Blood agars were incubated at 35 °C in the air for 24 hours, and grown colonies were identified by standard methods[14]. Inoculated enterococosel broth samples were also examined every 24 hours, and in case of darkening they were plated on enterococosel agar. Grown colonies were identifed using standard methods[14].

Results

The results of bacterial growth are shown in Table 1. Three samples treated with Nd:YAG laser at a power setting of 3.5 W were sterile 7 days after irradiation. In one of them, bacterial growth predominated by *Enterococcus* sp. occurred after 15 days, and the other two remained sterile after two weeks. In one sample pure culture of *Enterococcus* sp. had grown.

Irradiation with Nd:YAG laser at a power setting of 2.5 W did not sterilize any of the samples. During the first and second week after irradiation, both bacteria grew in great numbers.

Discussion

Staphylococcus aureus and *Enterococcus faecalis* are considered to be normal inhabitants of the infected endodontic space[16]. Due to the fact that the antibacterial property of lasers is mostly based on thermal effect, an important characteristic of bacteria used in laser studies is their thermoresistance. *Enterococcus faecalis*, although a nonsporiferous vegetative bacterium, is resistant to high temperatures[17]. *Staphylococcus aureus* is more heat-susceptible than *Streptococcus* spp.[9] This is in accordance with our findings in some samples where selective destroying of one bacterium species occurred; *Enterococcus faecalis* was the one that survived.

In their study Hardee *et al.*[6] achieved a reduction of CFU from $2.3x10^6$ to $0.6x10^6$ 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, they also report on a high increase in the temperature on the root, which made handling of the samples by fingers difficult. These results refer to possible periodontal injuries, which have been confirmed in the study by Bachall *et al.*[18] conducted on dogs. Irradiation of root canals with 3 W/30 s caused ankylosis, cemental resorption and major bone remodeling found on histologic analysis 30 days after treatment.

Antibacterial effects of the Nd:YAG laser were studied by Rooney *et al.*[8] in an experiment with a model consisting of small glass capillary tubes containing a measured

Tablica 1. Growth of bacteria (species and CFU/ml) in tooth samples upon laser exposure

Sample No.	Nd: YAG laser 3.5 W		Nd: YAG laser 2.5 W	
	7 days	15 days	7 days	15 days
1	SA+EF $>10^6$	SA+EF $>10^6$	SA+EF $>10^6$	SA+EF $>10^6$
2	SA+EF $>10^6$	SA+EF $>10^6$	SA+EF $>10^6$	SA+EF $>10^6$
3	Sterile	Sterile	SA+EF $>10^6$	SA+EF $>10^6$
4	SA+EF $>10^6$	SA+EF $>10^6$	SA+EF $>10^6$	SA+EF $>10^6$
5	Sterile	SA 10^3+EF $>10^6$	SA+EF $>10^6$	SA+EF $>10^6$
6	SA+EF $>10^6$	SA+EF $>10^6$	SA+EF $>10^6$	SA+EF $>10^6$
7	Sterile	Sterile	SA+EF $>10^6$	SA+EF $>10^6$
8	SA+EF $>10^6$	SA+EF $>10^6$	SA+EF $>10^6$	SA+EF $>10^6$
9	EF $>10^6$	EF $>10^6$	SA+EF $>10^6$	SA+EF $>10^6$
PC	SA+EF $>10^6$	SA+EF $>10^6$	SA+EF $>10^6$	SA+EF $>10^6$
NC	Sterile	Sterile	Sterile	Sterile

SA, *Staphylococcus aureus*; EF, *Enterococcus faecalis*; PC, positive control; NC, negative control

amount of bacterial broth. Using a fixed volume of black Suomi ink, they achived greater bactericidal effect with the energy of 25 J than when no dye was applied at the energy of 60 J.

This study demonstrated that although the dye was used, the specimens received a total amount of energy from 102.5 J to 156.5 J in only five cycles with a period of 15 seconds for cooling[15]. A sterilization effect was determined in only 3 root canals, especially in those root canals which were exposed to the higher energy levels and therefore had the possibility to induce damage to the periradicular tissue. Comparing this result with those of Rooney *et al.*[8], this discrepancy could be attributed to the difference between the experimental models. Morphologic variations of the extracted teeth, such as curved root canal and lateral and accessory root canals, dentinal tubules, etc., are suitable sites for bacteria to hide and become inaccessible to the laser beam. Although the use of the fiber optic delivery system and rotational movements improves delivery effects at such sites, the chance for the laser beam to reach the majority of bacteria is small due to the beam collimation.

The experiments by Moritz *et al.*[17] with Nd:YAG laser *in vivo* produced encouraging results for the reduction of bacteria in the root canal. With laser beam repeated five times for 10 s with a recovery period of 20 s, 1.5 W and repetition rate of 15 pulses/s, they managed to achieve a 50% reduction of bacteria specimens in their first attempt. Calculations yielded the highest log-ratio for *Streptococcus* spp. and *Staphylococcus* spp. of 3.9 and 4.32, respectively.

In these studies, the material for bacterial sampling was collected by irrigating the canal with saline and inserting a sterile paper point. This method allowed the bacteria to adhere to the root canal surface and hide in dentinal tubules, thus they were not present in the representative material. This is consistent with the low recovery rate of spores of up to 25%, recorded by Hardee *et al.*[6] in their control sample upon flushing of inoculated canals. In our study, the samples were placed in vials with brain-heart broth immediately upon lasing procedure, in order to include the hidden and adhered bacteria in the sample and to avoid dryness of the teeth. Moreover, the duration of the experiment (15 days) made possible the recovery of bacteria normally expected in clinical conditions.

The results of the present study are in accordance with the report of Barbakow *et al.*[19], who found that Nd:YAG laser did not significantly remove smear layer and debris. Dentin recrystallization was only achieved in the apical portion of the canal. Thus, the bacteria could easily remain hidden in the smear layer and debris, thus being inaccessible to the direct action of the laser beam.

In conclusion, the Nd:YAG laser with the parameters used in this study did not significantly reduce the number of bacteria in root canals.

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Sažetak

ANTIBAKTERIJSKI UČINCI Nd:YAG LASERA U UZORCIMA KORIJENSKOG KANALA: STUDIJA *IN VITRO*

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Svrha rada bila je ispitati antibakterijski učinak Nd:YAG lasera u korijenskim kanalima rabeći bakterijsku suspenziju *Enterococcus faecalis* i *Staphylococcus aureus*. Dvadesetdva jednokorijenska zuba su instrumentirana, sterilizirana te inokulirana bakterijskom suspenzijom koncentracije 10^6 CFU/ml u moždano-srčanom bujonu. Uzorci su obasjavani Nd:YAG laserom: jedna polovica uzoraka snagom od 3,5 W, a druga polovica snagom od 2,5 W, frekvencijom laserske zrake od 40 Hz. Uzorci su obasjavani u pet ciklusa u trajanju od 15 sekunda sa stankama za hlađenje od 15 sekunda. Nakon obasjavanja uzorci su smješteni u bočice s bujonom, te je CFU/ml određen nakon jednog odnosno dva tjedna. Laserske zrake snage 3,5 W sterilizirale su samo tri uzorka u prvom tjednu. U jednom od tih uzoraka u drugom su tjednu narasle bakterije, uglavnom roda *Enterococcus faecalis*, dok je u drugom uzorku narasla čitava kultura *Enterococcus faecalis*. Obasjavanje Nd:YAG laserskom zrakom snage 2,5 W nije steriliziralo niti jedan uzorak. Nd:YAG laser s parametrima rabljenim u ovom istraživanju nije značajno smanjio broj bakterija u uzorcima korijenskih kanala.

Ključne riječi: *Liječenje korijenskog kanala – metode; Laseri – terapijska primjena; Priprava korijenskog kanala – instrumentarij; Šupljina zubne pulpe – mikrobiologija; Dezinfekcija – metode*