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ORIGINAL ARTICLE

Comparison of two diode lasers on bactericidity in root canals—an in vitro study

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Abstract This in vitro study compares two 810-nm and 940-nm diode lasers on bacterial kill in root canals of extracted human teeth and shows the clinical relevance of different treatment modalities. Ninety root canals of single-rooted human teeth were prepared up to ISO 70, steam sterilized, and assigned to two test groups (810 nm, 940 nm) and one control group. Following an initiatory experiment in which access opening of root canals and surrounding cavity were excluded from irradiation in the main experiment, 60 teeth were inoculated with 2 μl of either *Escherichia coli* or *Enterococcus faecalis* suspension. Laser irradiation was performed, additionally including access opening of root canals and surrounding cavity in the laser treatment. Excluding access opening of root canals and surrounding cavity from the laser treatment, the diode laser achieved an average bacterial reduction of *Escherichia coli* of 76.06% (810 nm) and 68.15% (940 nm), while including access cavities showed an average bacterial reduction of *Escherichia coli* of 97.84% (810 nm) and 98.83% (940 nm) and an average bacterial reduction of *Enterococcus faecalis* of 98.8% (810 nm) and 98.66% (940 nm). Diode laser wavelengths are effective in endodontic therapy. It seems to be clinically relevant that additional irradiation of the access cavity produces significantly better bactericidal results.

Keywords Diode laser · Endodontology · Bacterial kill

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Introduction

Successful endodontic treatment is a necessary prerequisite in the long-term conservation of non-vital teeth. However, conventional root canal preparation and rinsing solutions cannot always eliminate the remaining bacteria in the root canal or dentin tubules sufficiently.

A central issue of conventional endodontic treatment is the depth of penetration reached by disinfecting procedures. Bacteria colonize the dentinal tubules up to 1,000 μm [1], whereas conventional rinsing solutions penetrate only about 100 μm [2] due to their surface tension.

Different laser wavelengths have been introduced in endodontic treatment in the past years. Near-infrared laser light reaches dentin layers to a depth>1,000 μm. Even this special group of lasers shows very different effectiveness on bacterial kill in root canals [3, 4].

In spite of the fact that laser light is weakened, the bactericidal effect is maintained because of the fact that enamel prisms and dentinal tubules act as light conductors [5, 6, 7].

The aim of this in vitro study was to compare two diode lasers with 810-nm and 940-nm wavelength on bacterial kill in root canals of extracted human teeth and to show the clinical relevance of including the surrounding cavity in the laser treatment.

Materials and methods

Ninety single-rooted, freshly extracted human teeth without any lesions in the apical area with formed and closed root apex were stored in physiological saline solution until used for the experiment. The clinical tooth crown had to be intact and the teeth had to have had no former endodontic treatment.

The trepanation with a diamond burr (Henry Schein, Vienna, Austria) was followed by an orthograde endodontic treatment in step-back technique up to ISO 70 (K-Files, Henry Schein, Vienna, Austria). Working length was determined with Raypex ("Raypex 5", VDW GmbH, Munich, Germany). During preparation, the root canals were rinsed with physiological saline solution continuously.

In order to guarantee a sterile setting, the samples were autoclaved for 30 min and 134°C temperature.

After steam sterilization, the teeth were assigned to two test groups, one for each laser wavelength. Two diode lasers of 810-nm (Biolase®, "ezlase 810 Biolase"; Biolase Technology Inc., San Clemente, CA) and 940-nm wavelength ("ezlase 940 Biolase", Biolase) were tested for their ability to remove bacteria from prepared root canals.

Escherichia coli (ATCC 10536) and *Enterococcus faecalis* (ATCC 29212) were used as germs. After cultivation on Columbia-Agar-Bases (BioMérieux, Marcy l'Etoile, France, Charge number: 819293701), the germs were identified with standard practice and inoculated in bouillon. The suspension had a concentration of 10^8 CFU/ml *Escherichia coli* and 10^9 CFU/ml *Enterococcus faecalis*. The storing was in liquid nitrogen. For each germ, one test tube was defrosted. *Escherichia coli* and *Enterococcus faecalis* had already been researched in detail and delivered reliable and reproducible results in many studies [8, 9, 10].

In an initiatory experiment, 30 root canals, prepared as described above, were inoculated with 2 μl of *Escherichia coli* suspension of defined concentration.

Each prepared root was inoculated with 2 μl germ suspension by means of an Eppendorf pipette from a coronal direction and incubated in an incubator ("BD 23", Binder GmbH, Tuttlingen, Germany) with 36.2 ± 0.7° temperature and a relative humidity of 46–56% for 2 h to guarantee an adequate penetration of the ultrastructure of the root canals.

Afterwards, laser energy was delivered into the root canals of the two laser groups (ten teeth per group) by means of a flexible fiber, 200-μm-diameter, single-use fiber (Biolase). The laser tips were inserted until 1 mm above the apex and removed in slow, helical movements, in an apical-coronal direction, taking care that each part of the canal wall was reached.

Each root canal was treated in five irradiation cycles consisting of 5-s irradiation followed by a 20-s break.

Access opening of root canals and surrounding cavity were excluded from irradiation. The third group served as a control remaining without laser irradiation.

The following parameters were defined at the beginning:

- ezlase™ 810 Biolase: 4.5 W power, pulse interval 0.10 ms, pulse length 0.05 ms, energy start 29.6 J, average power 1.5 W;
- ezlase™ 940 Biolase: 4.5 W power, pulse interval 0.10 ms, pulse length 0.05 ms, energy start 31.8 J, average power 1.5 W;

The samples of the control group were treated equally except for the activation of the laser. To guarantee the same conditions, the inactive laser tip was inserted in the root canal.

In the main experiment, 60 steam-sterilized teeth were assigned to two test groups, one for each laser wavelength. The root canals were inoculated with 2 μl of either *Escherichia coli* or *Enterococcus faecalis* suspension of defined concentration. Subsequently, the teeth were incubated for 2 h. Afterwards, the laser energy was delivered into the root canals as described above, but including access opening of root canals and surrounding cavity in the laser treatment (ten teeth for each wavelength and germ). We used the same treatment sequence as described before (five times irradiation for 5 s with 20-s breaks in between).

After the laser treatment, all samples were put in sterile test tubes and filled with 1 ml of physiological saline solution. The samples were put on an automatic shaker (Variomag Teleshake, INHECO, Martinsried, Germany) for 15 min to guarantee an almost complete dilution of the present bacteria.

From this 1-ml eluate, logarithmic dilution series were produced, 20 μl of each dilution were put on Columbia-Agar-Bases (BioMérieux, Marcy l'Etoile, France, Charge number: 819293701).

The samples were incubated under the conditions mentioned above. Afterwards, the colony-forming units (CFU) were counted and the reduction of bacteria was calculated. Statistical analysis was done by Mann–Whitney *U* test, and Kruskal–Wallis test was applied to calculate significance.

The investigation was accepted by the Ethic Commission of Vienna Medical University (No. 058/2009).

Results

The average concentration of *Escherichia coli* in the control group of the initiatory experiment the blank value was 2.85×10^6. This comes up to a reduction of bacteria of two logarithmic steps compared to the initial suspension (10^8). This decrease is due to inoculation and incubation. Excluding access opening of root canals and surrounding

cavity from the laser treatment, the diode laser with 810-nm wavelength achieved an average bacterial reduction of *Escherichia coli* of 76.06% to a value of 6.8227×10^5 and 68.15% to a value of 9.076×10^5 with 940-nm wavelength (Fig. 1).

Including access opening of root canals and surrounding cavity in the laser treatment, a blank value of 1.27×10^6 was found. The diode laser with 810-nm wavelength achieved an average bacterial reduction of *Escherichia coli* of 97.84% to a value of 2.745×10^4 and 98.83% to a value of 1.48×10^4 with 940-nm wavelength (Fig. 2).

Examinations showed an average bacterial reduction of *Enterococcus faecalis*, compared to a blank value of 1.403×10^6 with 810-nm diode laser of 98.8% to a value of 1.682×10^4 and 98.66% to a value of 1.875×10^4 with 940-nm wavelength (Figs. 3, 4).

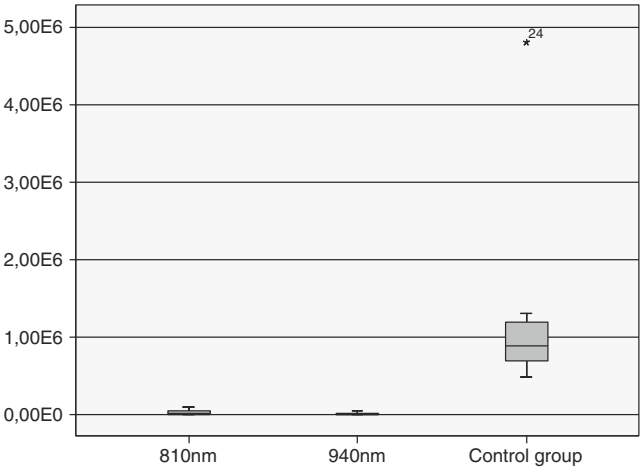

Fig. 2 Main experiment: *Escherichia coli*, ordinate depicting the total bacterial count after laser irradiation including the access cavity

Discussion

The use of modern laser technology in endodontic therapy has the great benefit of reaching areas that are not accessible to conventional rinsing solutions.

It has already been demonstrated that diode lasers provide satisfactory bactericidal effects compared to the neodym: yttrium-aluminium-garnet (Nd:YAG) laser [11, 12].

The initial bacterial reduction of two logarithmic steps as described in the Results section can be explained by a deficient mixing or the fact that the rest of the bacterial suspension remained in the tip of the pipette.

Because of the fact that *Enterococcus faecalis* tends to form pairs and chains, it cannot be excluded that the elution procedure was not complete. Furthermore, the penetration of the bacteria in the dentine tubules could have impeded the elution of the bacteria. The level of diffusion of bacteria into dentine tubules was not measured but can be estimated based on prior literature [1, 2]. Because the smear layer was not removed with EDTA, the diffusion could have been limited.

Enterococcus faecalis is known for its ability to form intra- and extraradicular biofilms, further complicating the elution [13, 14, 15]. Transferred to clinical cases, this would result in a resistance to therapy or a long-term failure of endodontic therapy.

Moritz et al. have already demonstrated the importance of the structural configuration of the cell wall concerning the sensitivity to laser irradiation. Gram-negative bacteria are affected immediately, whereas Gram-positive bacteria need to be irradiated repeatedly in order to be damaged lethally. The extent of damage is proportional to the applied energy [16].

Gurbuz et al. showed by comparing several rinsing solutions and subsequent irradiation with the Nd:YAG laser that there are significant differences in the mineral content and the morphology of the dentine surface [17].

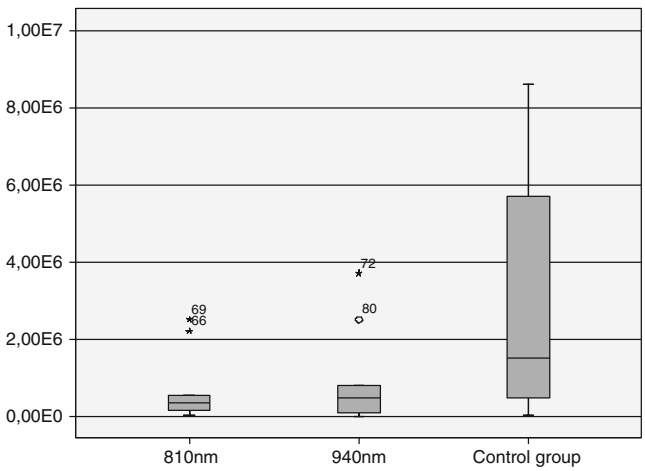

Fig. 1 Initiarory experiment: Escherichia coli, ordinate showing the total bacterial count after laser irradiation excluding the access cavity

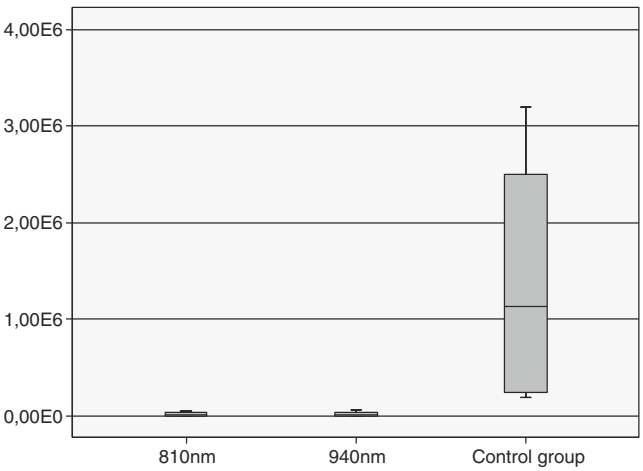

Fig. 3 Main experiment: *Enterococcus faecalis*, ordinate showing the total bacterial count after laser irradiation including the access cavity

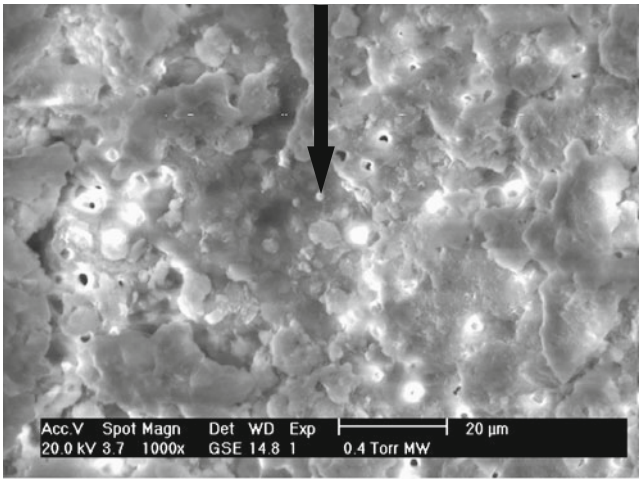

Fig. 4 In order to evaluate a morphologic correlate, the samples were split with a chisel (in order to avoid grinding artefacts) and analyzed with a scanning electron microscope at the Technical University of Vienna. Neither heat damage nor debris could be detected. Sporadically, bacterial residua could be identified (as marked in the image below, magnification 1000×)

The Nd:YAG laser as well as the diode laser change the morphology of the dentine surface, the changes are more distinct after Nd:YAG laser treatment. By means of a SEM examination (scanning electron microscope) a fusion and a new consolidation of the dentine surface could be verified. The changes after diode laser treatment were more uniform compared to Nd:YAG laser treatment according to de Moura-Netto et al.[18].

Marchesan et al. showed that dentine permeability after laser irradiation is directly dependent on the last rinsing solution used [19]. Consequently, further investigations should be conducted in order to adapt the endodontic rinsing solution to the wavelength used.

Both wavelengths used in the experiment achieved satisfactory bactericidal effects and are suitable tools in a modern state-of-the-art endodontic therapy, even covering the spectrum of endodontically problematic germs as *Enterococcus faecalis*.

Conclusions

In conclusion, the 810-nm as well as the 940-nm wavelength diode laser can be used for effective endodontic therapy. Further in vivo studies should be conducted, however. Especially in combination with conventional treatments, diode laser technology can be regarded as an additional possibility to guarantee successful endodontic treatment. The fact that the bacterial reduction in teeth including access opening of root canals and surrounding cavity in the laser treatment showed significantly better results can be interpreted as clinically relevant.

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