

Original Article

Bactericidal Effects of 2.94 μm and 1.67 μm Laser

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Received 4 December, 2009/Accepted for publication 9 June, 2010

Abstract

The bactericidal effects of lasers with wavelengths of 1.67 and 2.94 μm on cariogenic *Streptococcus mutans* were investigated. Temperature during irradiation was also measured to determine the mechanism underlying the bactericidal effects of the lasers. An aliquot of 2 μl cell suspension of *S. mutans* JC-2 strain was placed on anhydrous quartz or dentin plate, covering an area of approximately 5.0 mm in diameter to a depth of approximately 0.1 mm. Cell suspension was then irradiated at a power of 0.8 W (3.1 J/cm²) at a rate of 40 pps for 30 sec. After irradiation, the plate was put into a bottle containing PBS and vigorously vorticated. Solution was serially diluted and inoculated on MS agar. After incubation anaerobically for 72 hr, colony forming units on the agar were counted. The experimental group, the number of bacteria decreased significantly compared to the control group under all conditions. No significant differences were observed in effect of wavelength or plate on bactericidal activity. In conclusion, laser irradiation at a wavelength of 1.67 μm for 30 sec showed a bactericidal effect on *S. mutans*, suggesting that this wavelength is more useful than 2.94 μm due to greater tissue penetration.

Key words: *Streptococcus mutans*—Er:YAG laser—Photochemical—Caries—Bactericidal effect

Introduction

The current trend in caries therapy is the

minimal intervention (MI) strategy, in which teeth are cut as minimally as possible using a laser and chemical dissolution, with strength-

ening of remaining teeth. Carious dentin that is infected by bacteria and cannot recalcify is completely removed and dentin suitable for recalcification is retained and repaired. To address the problem of residual caries-inducing bacteria after caries therapy, carious dentin can be sealed with an adhesive resin containing the antibacterial monomer methacryloyloxydodecylpyridinium bromide (MDPB); this procedure is referred to as modified sealed restoration^{11,12)}. The effect of photodynamic therapy (PDT) on bacteria has also been reported⁹⁾.

Lasers have been used clinically in dental practice for various purposes, including tooth cutting, washing in root canals, scaling, and pain relief in soft tissues^{14,15)}; and many studies have reported the bactericidal effects of lasers.^{2,4-6,8-10,13,18)} However, lasers have different properties depending on wavelength; for example, the Nd:YAG laser has high tissue permeability and is able to affect deep tissue, whereas the Er:YAG laser has good absorption properties in water and acts on the surface of tissues.

The Er:YAG laser provides effective dentin cutting with less undesirable noise and vibration, and is used clinically as a caries cutter subsequent to use of an air turbine^{7,17)}. The smear layer is removed from the dentin surface by irradiation with an Er:YAG laser, thereby eliminating one of the conditions that support residual caries-inducing bacteria. Since the energy of the Er:YAG laser is focused on the tissue surface, it can sterilize surface dentin but not the deeper tissue; therefore, sterilization of the remaining dentin requires irradiation using a laser with higher tissue permeability. This requirement suggests that a dual-wavelength laser system would be useful for caries therapy. We have investigated the performance of such a system for cutting soft and hard tissues, together with possible adverse effects on pulp¹⁾. Using a system providing simultaneous lasers with wavelengths of 1.67 μm and 2.94 μm , we showed that the 1.67 μm laser had higher dentin permeability than the 2.94 μm laser.

In this study, we hypothesized that the dual-

wavelength laser system would treat carious dentin with its 2.94 μm laser, the same wavelength as that of the Er:YAG laser, while simultaneously decreasing caries-inducing bacteria in the remaining tooth with its 1.67 μm laser, which has high dentin permeability. The efficacy of this approach for caries therapy was assessed by determining the bactericidal effects of the two lasers on *Streptococcus mutans*. The temperature during irradiation was also measured to investigate the mechanism underlying the bactericidal effects of the lasers.

Materials and Methods

1. Materials

The bacterial strain used in the study was *S. mutans* JC-2 provided by the Department of Microbiology, Tokyo Dental College. Anhydrous quartz and dentin plates were used in the irradiation experiments. The anhydrous quartz plates were parallel substrate disc plates (Sigma Koki Co., Ltd.) of 3 mm in thickness and 30 mm in diameter; these plates have no laser absorption properties. The dentin plates were made from root dentin from bovine mandibular anterior teeth. Cryopreserved bovine teeth were thawed, the crown cut at the neck, and the root divided along the labiolingual plane and polished with a grinding wheel (#400, #800) followed by water-proof abrasive paper (#800, #1200). After polishing, the root was cut into pieces of approximately 1 mm in thickness with an area of 7 \times 7 mm. All plates were sterilized in an autoclave at 121 $^{\circ}\text{C}$ for 15 to 20 min after ultrasonic cleansing. The dentin plates were stored in sterile purified water until irradiation.

2. Laser system

A tunable laser system (Dual Wave Length Laser Equipment with Optical Parametric Oscillator, J. Morita Mfg. Corp., Kyoto, Japan) was used in the study. With this system, you can simultaneously generate separate lasers wavelengths of 1.67 μm and 2.94 μm by changing the mixing ratio. A prototype version of the system was used to guide the laser beam to

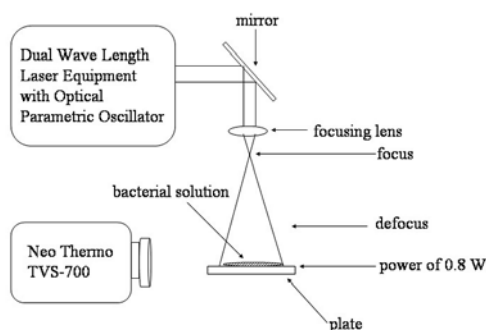


Fig. 1 Schematic of experiment

the irradiation site using a single-joint mirror system, with use of a lens to defocus the beam over an irradiation area of 5.0 mm in diameter on the surface of a bacterial solution on a quartz or dentin plate.

3. Irradiation conditions

An aliquot of 2 μl *S. mutans* suspension was placed on a plate such that it covered an area of about 5.0 mm in diameter to a depth of approximately 0.1 mm (Fig. 1). The solution was then irradiated at a power of 0.8 W (3.1 J/cm²) and a rate of 40 pps for 30 sec. After irradiation, the plate was put into a specimen bottle and 1 ml phosphate buffered saline (pH 7.2, PBS) added. The mixed solution was diluted in a 10 fold series. Each 100 μl sample was plated on Mitis-Salivarius (MS) agar (Becton Dickinson Microbiology System, Cockeysville, MD), spread evenly with a conrage stick, and cultured anaerobically for 72 hr, after which the bacterial colony forming units (CFUs/ml) on the agar plates were counted. The plate material and irradiation conditions in separate experiments ($n=8$ in each experiment) were as follows: anhydrous quartz + 1.67 μm ; anhydrous quartz + 2.94 μm ; dentin + 1.67 μm ; and dentin + 2.94 μm . Colony formation was also determined on non-irradiated control plates. The bactericidal effect was compared between groups using the Dunnett test.

4. Temperature measurements

The temperature of the anhydrous quartz

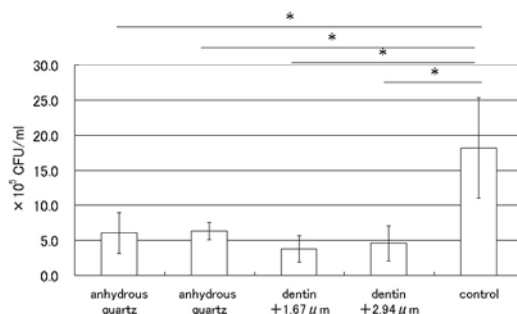


Fig. 2 Mean numbers of surviving CFUs (/ml) (* $p<0.05$) Number of bacteria decreased significantly compared to the control group under all conditions. See edge of abstract.

or dentin plate and the temperature of the bacterial solution on the plate were measured before and at 30 sec after irradiation at 1.67 μm or 2.94 μm . Measurements were made using an instrument for infrared thermography (Neo Thermo TVS-700, Nippon Avionics Co., Ltd.) installed on a tripod. The recorded thermographs were analyzed using thermal image analysis software (PE Professional, Nippon Avionics Co., Ltd.).

Results

1. Bactericidal effect of laser irradiation

S. mutans cells plated onto the anhydrous quartz and dentin were significantly killed by laser irradiation with the tunable laser system ($p<0.05$, Fig. 2). A comparison of CFUs following irradiation at different wavelengths showed no significant difference between 1.67 μm and 2.94 μm irradiation on quartz or dentin plates. Similarly, there was no significant difference between CFUs on quartz and dentin plates following irradiation at 1.67 μm or 2.94 μm .

2. Changes in temperature due to laser irradiation

The temperature of the plate ranged from 25.9 $^{\circ}\text{C}$ to 27.6 $^{\circ}\text{C}$ at room temperature (25 $^{\circ}\text{C}$). The plate temperature of the anhydrous quartz increased by 1.2 \pm 0.3 $^{\circ}\text{C}$ and 0.2 \pm 0.2 $^{\circ}\text{C}$ by

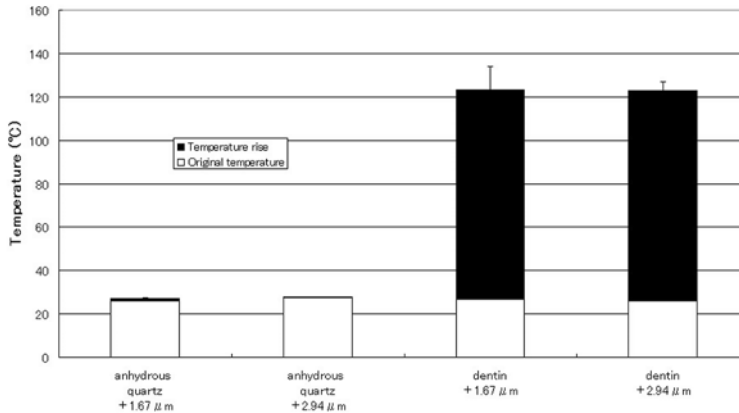


Fig. 3 Temperature change of plate after laser irradiation

Almost no increase in temperature of anhydrous quartz at 1.67 μm or 2.94 μm, but temperature of dentin plates increased to more than 90°C at both wavelengths.

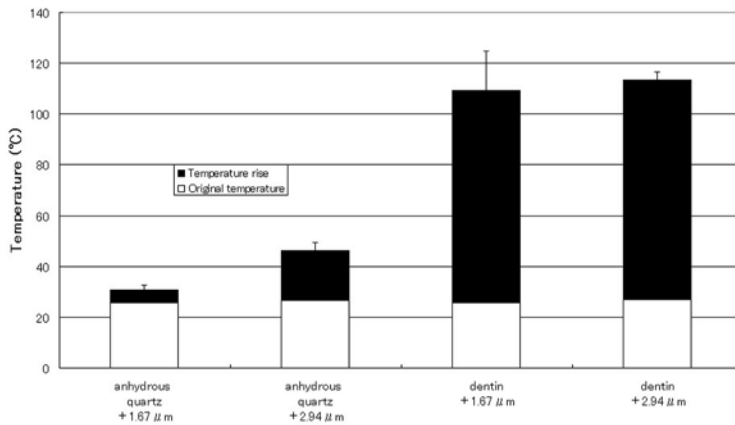


Fig. 4 Temperature change of bacterial solution after laser irradiation

Temperature of bacterial suspension on anhydrous quartz increased by approximately 5°C by 1.67 μm and 20°C by 2.94 μm. Temperature of bacterial suspension on dentin plate increased to more than 80°C at both wavelengths.

1.67 μm and 2.94 μm laser irradiation, respectively. In contrast, the plate temperature of the dentin increased by $96.5 \pm 10.6^\circ\text{C}$ and $97.1 \pm 4.0^\circ\text{C}$ by 1.67 μm and 2.94 μm laser irradiation, respectively (Fig. 3). The bacterial suspension was incubated at 37°C before being dropped onto the plate and irradiated. The temperature of the bacterial suspension on the hydrous quartz increased to $5.1 \pm 1.7^\circ\text{C}$ and $19.7 \pm 3.2^\circ\text{C}$ by 1.67 μm and 2.94 μm laser irradiation, respectively. The temperature of

the bacterial suspension on dentin increased to $83.9 \pm 15.5^\circ\text{C}$ and $86.5 \pm 3.1^\circ\text{C}$ by 1.67 μm and 2.94 μm laser irradiation, respectively (Fig. 4).

Discussion

Recent studies have been conducted to investigate the bactericidal effects of laser irradiation, since conventional methods do not

provide sufficient sterility for caries, periodontal disease or infected root canals^{2,4-6,8-10,13,18)}. In this study, *S. mutans* was selected as the target from the perspective of prevention of secondary caries.

Wilson¹⁹⁾ demonstrated that the bactericidal effects of laser irradiation depend on energy density, irradiation time and bacterial conditions. Moritz *et al.*¹⁰⁾ used Nd:YAG, Ho:YAG and Er:YAG lasers at powers of 0.8 and 1.5 W and a repeat rate of 10 Hz for 5 sec, repeated 5 times at 15 sec intervals to irradiate *Escherichia coli* and *Enterococcus faecalis* in an investigation of the bactericidal effects of laser irradiation for infected root canals. Slight differences in bactericidal effects were observed between lasers at a low energy density of 0.8 W, whereas no differences were found among lasers operating at 1.5 W. This suggests that a high energy density has a strong bactericidal effect and obscures differences between laser wavelengths; therefore, for comparison of effects between wavelengths, it is desirable to irradiate with a laser operating at a low energy density.

In this study, irradiation was conducted at a power of 0.8 W (3.1 J/cm²) and a repeat rate of 40 pps for 30 sec, with the power and irradiation time determined based on preliminary results and previous studies. The repeat rate of 40 pps was chosen because pulse irradiation was expected to cause a smaller increase in temperature than continuous-wave irradiation.

Türkün *et al.*¹⁸⁾ used irradiation with an Er,Cr:YSGG laser of wavelength 2.78 μ m (close to the wavelength of 2.94 μ m used in the current study) for 5 sec, repeated 5 times at 15 sec intervals to investigate bactericidal effects on *S. mutans*. At powers of 0.75 W and 1.0 W, approximately 94% and 99% of bacteria were killed and no significant difference was found between chlorhexidine gluconate-treated bacteria and irradiated bacteria, suggesting a marked bactericidal effect of laser irradiation. Ando *et al.*²⁾ used an Er:YAG laser with a wavelength of 2.94 μ m to irradiate *Porphyromonas gingivalis* and reported bactericidal effects of 68% and 83% at 7.1 and 10.6 J/cm², respectively. Hence, this study was conducted using

a similar laser wavelength, bacterial strain and irradiation time as those in Türkün *et al.*¹⁸⁾ and Ando *et al.*²⁾, and similar bactericidal effects were obtained.

Ando *et al.*²⁾ investigated the relationship between energy density and the bactericidal effect of an Er:YAG laser on *P. gingivalis* periodontal bacteria, and reported that laser irradiation at 1.8–3.5 J/cm² had a bactericidal effect of 0–9%, whereas irradiation at 7.1–10.6 J/cm² had an effect of 68–83%. The surface of colonies irradiated at 1.8–3.5 J/cm² showed slight whitening, but the surface at 7.1–10.6 J/cm² exhibited transpiration from inner colonies. These results indicate that a higher energy density has a stronger bactericidal effect. Therefore, if a contact tip of 200 to 600 μ m in diameter had been attached in the current study, thereby increasing the energy density by approximately 600 to 70 fold, it is likely that the bactericidal effect would have been further enhanced.

Previous studies of bactericidal effects of laser irradiation have used a device with an attached contact tip and have been conducted at high energy density. Contact tips ranging from 200 to 600 μ m in head diameter are commonly used in general caries, periodontal and dental therapy. The energy density in this study was 3.1 J/cm², but with contact tips of 600, 400 and 200 μ m, the energy density was estimated to be increased by approximately 600 to 70 fold to 212, 477 and 1,910 J/cm², respectively. Moritz *et al.*¹⁰⁾ and Schoop *et al.*¹³⁾ used contact tips of 400 μ m with energy densities of 1,592 to 2,986 J/cm² and 656 to 1,711 J/cm², respectively; Jelínková *et al.*⁶⁾ used contact tips of 600 and 700 μ m with energy densities of 2,653 and 780–2,340 J/cm², respectively; and Mehl *et al.*⁹⁾ used a contact tip of 375 μ m with energy densities ranging from 679 to 2,717 J/cm². These data suggest that previous studies have been conducted at energy densities of approximately 200 to 900-fold higher than that in this study. Therefore in our study, the contact tip of the laser was detached and the laser beam defocused over an irradiation area of 5.0 mm in diameter using a lens in order to obtain even irradiation of

the bacterial solution.

In this study, the temperature of the bacterial solution during laser irradiation was determined to investigate whether the bactericidal effect was photothermal. The temperature of the bacterial suspension during laser irradiation at $2.94\mu\text{m}$ increased by approximately 20°C and 87°C on the anhydrous quartz and dentin plates, respectively, showing a difference in temperature between these plates of approximately 67°C . This difference was due to the different absorption properties of the plates with a laser of $2.94\mu\text{m}$. Anhydrous quartz does not absorb laser energy, and therefore the laser only effected water in the bacterial solution on the anhydrous quartz plate. In contrast, with a dentin plate, the laser energy is absorbed by both the bacterial solution and the plate, causing the dentin temperature to increase and inducing a further increase in the temperature of the bacterial solution. However, despite these differences, there was little difference in the bactericidal effects of the $2.94\mu\text{m}$ laser on the anhydrous quartz (65.1%) and dentin (74.8%) plates.

Different lasers are thought to have different respective bactericidal mechanisms. For example, Grönqvist *et al.*⁵⁾ showed that the bactericidal effect of an Nd:YAG laser on *Staphylococcus epidermidis* was photothermal, whereas Folwaczny *et al.*⁴⁾ reported that the bactericidal effect of an excimer laser was photochemical and independent of temperature. Therefore, lasers may kill bacteria both photothermally and photochemically, with the specific effect depending on wavelength and energy density. The bactericidal effect of a laser at $2.94\mu\text{m}$ may be due to two mechanisms of action. The Er:YAG laser is highly absorbed by water and causes physical breakdown of hard tissues, resulting in transpiration. Ando *et al.*²⁾ showed that Er:YAG laser irradiation of water-rich bacteria and bacterial colonies caused the water inside the bacteria to immediately transpire, leading to a "micro-explosion" and physical breakdown of the bacterial cell structure. Using an Er:YAG laser at $35.4\text{mJ}/\text{cm}^2$ (100 mJ, 1 pps), Yamaguchi *et al.*²⁰⁾ observed removal of *E. coli*-derived

lipopolysaccharide (LPS) on the surface of tooth roots using a scanning electron microscope (SEM) and found that freeze-dried LPS has an absorption peak at $2.92\mu\text{m}$ and that 83.1% of the LPS was removed by Er:YAG laser irradiation.

The temperature of the bacterial solution during laser irradiation at $1.67\mu\text{m}$ increased by approximately 5°C and 84°C on the anhydrous quartz and dentin plates, respectively. The difference in temperature of about 79°C was due to the absorption properties of the plates with a laser of $1.67\mu\text{m}$. Anhydrous quartz has no laser absorption and the laser had only a small effect on the temperature of the bacterial solution. In contrast, the temperature of the dentin plate was increased by absorption of laser energy, thereby causing an increase in the temperature of the bacterial solution.

The temperature of the bacterial solution after laser irradiation in the dentin + $1.67\mu\text{m}$ group was approximately 110°C higher than the protein coagulation temperature at which transpiration can develop and a photothermal effect be expected. The temperature of the bacterial solution after laser irradiation in the anhydrous quartz + $1.67\mu\text{m}$ group was approximately 32°C , at which a photochemical effect is generally expected. However, the bactericidal effect was 66.7% in the anhydrous quartz + $1.67\mu\text{m}$ group and 79.3% in the dentin + $1.67\mu\text{m}$ group. The temperature measurements suggest a photothermal bactericidal effect, but no significant difference was observed between the plates. Therefore, these results suggest that the bactericidal effect of the laser at low energy density in this study did not depend on the increase in temperature caused by laser irradiation.

Regarding the laser at $2.94\mu\text{m}$, the temperature in the anhydrous quartz + $2.94\mu\text{m}$ group increased but did not reach 60°C , the temperature required for protein denaturation. In addition, amine and amide groups are able to absorb at this wavelength; therefore, the bactericidal effect was considered to be a photochemical effect. The laser at $1.67\mu\text{m}$ is absorbed by amide groups, and

based on the above conclusion that the bactericidal effect of this laser was not photothermal, it appears likely that the 1.67 μ m laser also had a direct photochemical effect on the bacteria.

Our results suggest that the bactericidal effects of the lasers at 2.94 μ m and 1.67 μ m were photochemical due to the absorption properties of amine and amide groups, respectively.¹⁶⁾

Conclusion

Our results indicate that lasers with wavelengths of 1.67 μ m and 2.94 μ m had similar bactericidal effects on *S. mutans* on anhydrous quartz plates. These results also suggest that these effects were photochemical, rather than photothermal.

Acknowledgements

We would like to express our sincere gratitude to the staff of the Department of Operative Dentistry, Tokyo Dental College, for their advice. In addition, we express our appreciation to Professor Katsuji Okuda, Department of Microbiology, Tokyo Dental College, for his cooperation and advice. We would also like to thank J. Morita Mfg. Corp.

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