# Antimicrobial Efficacy of Semiconductor Laser Irradiation on Implant Surfaces

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***Purpose:*** *This study was conducted to investigate the antimicrobial effect of an 809-nm semiconductor laser on common dental implant surfaces.* ***Materials and Methods:*** *Sandblasted and acid-etched (SA), plasma-sprayed (TPS), and hydroxyapatite-coated (HA) titanium disks were incubated with a suspension of S sanguinis (ATCC 10556) and subsequently irradiated with a gallium-aluminum-arsenide (GaAlAs) laser using a 600-µm optical fiber with a power output of 0.5 to 2.5 W, corresponding to power densities of 176.9 to 884.6 W/cm$^2$. Bacterial reduction was calculated by counting colony-forming units on blood agar plates. Cell numbers were compared to untreated control samples and to samples treated with chlorhexidine digluconate (CHX). Heat development during irradiation of the implants placed in bone blocks was visualized by means of shortwave thermography.* ***Results:*** *In TPS and SA specimens, laser irradiation led to a significant bacterial reduction at all power settings. In an energy-dependent manner, the number of viable bacteria was reduced by 45.0% to 99.4% in TPS specimens and 57.6% to 99.9% in SA specimens. On HA-coated disks, a significant bacterial kill was achieved at 2.0 W (98.2%) and 2.5 W (99.3%) only (t test, $P < .05$). For specimens treated with CHX, the bacterial counts were reduced by 99.99% in TPS and HA-coated samples and by 99.89% in SA samples.* ***Discussion:*** *The results of the study indicate that the 809-nm semiconductor laser is capable of decontaminating implant surfaces. Surface characteristics determine the necessary power density to achieve a sufficient bactericidal effect. The bactericidal effect, however, was lower than that achieved by a 1-minute treatment with 0.2% CHX. The rapid heat generation during laser irradiation requires special consideration of thermal damage to adjacent tissues.* ***Conclusion:*** *No obvious advantage of semiconductor laser treatment over conventional methods of disinfection could be detected in vitro.* INT J ORAL MAXILLOFAC IMPLANTS 2003;18:706–711

**Key words:** *dental implants, disinfection, laser irradiation, titanium*

In addition to numerous local and systemic etiologic factors, peri-implant bacterial infection resulting in inflammation of the surrounding soft tissues or even loss of implant-supporting bone can compromise clinical implant success. In regenerative peri-implantitis therapy combined with autogenous bone grafting[1] or membrane placement,[2] painstaking cleaning of exposed and contaminated implant surfaces is one of the prerequisites for treatment success. A variety of mechanical and chemical cleaning regimens have been described.[3,4] The application of air-powder systems was reported to have the highest efficacy of all conventional cleaning procedures in vitro.[5]

Reports on laser-assisted decontamination of implant surfaces have been available, and the results are mostly favorable.[6–9] However, some lasers, such as the neodymium:yttrium-aluminum-garnet (Nd:YAG), the holmium:YAG (Ho:YAG), or the frequency-doubled Alexandrite laser, are not suitable for decontamination of titanium surfaces, since even at

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minimal energy fluences, considerable surface damage takes place.[10–13] Considering recent scientific results, surface decontamination in vitro is possible by means of the carbon dioxide ($CO_2$)[14] and erbium:YAG (Er:YAG)[15] lasers at power densities that do not adversely influence implant surface characteristics. The diode laser does not damage implant surfaces, even at relatively high power densities.[12,13] Although positive reports on the application of the diode laser in the conventional treatment of peri-implantitis have been published,[16] optimal irradiation parameters for decontamination of different implant surfaces with the 800-nm to 810-nm semiconductor require scientific investigation. Moreover, during laser irradiation of implant surfaces, potential thermal damage to adjacent tissues must be taken into account. In contrast to the Er:YAG laser,[17] application of both the $CO_2$ and the 809-nm diode laser at clinically relevant energy settings for surface decontamination may induce considerable heat generation in the peri-implant bone.[18] Temperatures over 47°C induce tissue damage in the bone[19] and must therefore be avoided during all surgical procedures, including laser-assisted surface cleaning. Clinical guidelines are therefore needed to ensure a sufficient cleaning effect without jeopardizing adjacent tissues.

The aim of this study was to evaluate the antimicrobial effect of the 809-nm diode laser on implant surfaces under simple and reproducible experimental conditions. Infrared thermography was used to visualize heat generation in the implant surface and the peri-implant bone during simulated laser decontamination.

## MATERIALS AND METHODS

### Titanium Disks

Test disks made of commercially pure titanium (cpTi) of a thickness of 1.5 mm and a diameter of 10 mm with 3 different surfaces (sandblasted/acid-etched [SA], titanium plasma-sprayed [TPS], and hydroxyapatite-coated [HA]; Friadent, Mannheim, Germany) served as substrates. Surface roughnesses ($R_a$) as indicated by the manufacturer were 2.2 µm (SA), 3.41 µm (TPS), and 2.0 µm (HA) with a standard deviation of approximately 20%.

### Target Microorganism and Incubation

*Streptococcus sanguinis* (ATCC 10556) was obtained commercially (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany). It was cultured on Columbia blood agar plates (Heipha Dr Müller, Heidelberg, Germany). The bacterial cells were washed 3 times with phosphate-buffered saline (PBS; pH = 7.2) and then suspended in PBS. For quantification of bacterial cell numbers present in suspensions, viable bacterial colony counts were determined. The mean concentration was $8\times10^8$ cells/mL. Prior to inoculation of the specimens, the solution was sonicated to disperse cell clumps.

**Fig 1** Standardized irradiation was carried out on a computer-controlled x-y translation stage. Movement of the fiber was performed in concentric circles from the center to the periphery of the disks. The radius of the circles was successively enhanced by 600 µm, which corresponded to the diameter of the fiber.

The disks were incubated using a technique described elsewhere.[15] Incubation time was 60 minutes at 37°C. Only disks of the same kind were incubated simultaneously. After incubation the disks were washed with PBS to remove non-adherent cells and subsequently irradiated at different power densities. Two control groups were formed. Specimens of the first control group were not treated at all, and specimens of the second control group were placed passively for 1 minute into a vial with a solution of 0.2% chlorhexidine digluconate (CHX).

### Laser

A gallium-aluminum-arsenide (GaAlAs) laser (λ = 809 nm) (Oralaser Voxx; Oralia, Konstanz, Germany) with a 600-µm optical fiber was used. For irradiation, the disks were mounted on a PC-controlled x-y translation stage, ensuring standardized irradiation of the specimens. The distance from end of the fiber to the surface of the specimens was kept constant at 0.5 mm. The angle of irradiation was 90 degrees (Fig 1). The movement was performed in concentric circles from the center to the periphery of the disks. The radius of the circles was successively enhanced by 600 µm, corresponding to the diameter of the fiber. The overall treatment time per specimen (lased area: 0.785 $cm^2$) was 60 seconds.

Prior to lasing, the average power output of the laser system was determined by means of an energy meter (Field Master GS; Coherent, Dieburg, Germany). Power output as measured at the end of the fiber was 0.5, 1.0, 1.5, 2.0, and 2.5 W (continuous-wave mode) with a deviation of below 5%, corresponding to a power density range of 176.9 to 884.6 W/cm$^2$.

### Bacterial Counts

After the respective treatments, the disks were placed into 10 mL of sterile PBS and were sonicated with a Branson Sonifier (Branson Ultrasonics, Danbury, CT) for 2 × 45 seconds at 75 W. After serial tenfold dilution, aliquots of each dilution (100 µL) were spread on Columbia blood agar plates. The samples were incubated for 48 hours at 37°C in an aerobic atmosphere and the number of colony-forming units (cfu) was determined. From this, the number of bacteria per sample was calculated.

### Statistical Analysis

For each respective surface, 8 specimens were irradiated at different power settings and compared to the 8 untreated specimens and the 8 specimens treated with CHX. Bactericidal efficacy of the treatment regimens was indicated in logarithmic steps. The *t* test was used to compare treatment groups with the control group at a significance level of 5%.

### Temperature Measurements

Five TPS stepped-cylinder implants (Frialit-2; Friadent, Mannheim, Germany) with a diameter of 3.8 mm and a length of 11 mm were placed into bone blocks cut from fresh pig femurs. The implant cavity was drilled on the edge of the bone and approximately one third of the cylinder was uncovered to determine surface temperatures. The block was placed into a water-filled heating circulator (Julabo MWB; Julabo Labortechnik, Seelbach, Germany) with only the area to be lased not being submerged. The system was stabilized at a temperature of 37°C to simulate in vivo thermal conductivity and diffusiveness of heat. The implants were irradiated in one spot to ensure reproducible test conditions. Temperature changes during irradiation were recorded using a shortwave thermocamera (AGEMA 470; Agema, Stockholm, Sweden) with a wavelength range of 2,500 to 5,000 nm and a standard lens (field of view 20 degrees; geometric resolution 3.9 mRad). Video images were analyzed semiquantitatively by means of application software (IRWIN 5.21; Agema).

## RESULTS

### Antimicrobial Efficacy

Cell counts in untreated control specimens on the TPS surface amounted to a mean of $2\times10^5$ and were comparable to those calculated on the HA disks (Tables 1 to 3). The number of cells on the SA specimens was approximately 1 log step lower.

In TPS and SA specimens, laser irradiation led to a significant bacterial reduction at all power settings. The antimicrobial effect increased with higher power density (Tables 1 and 2). Complete bacterial reduction could not be achieved in any of the specimens. Laser treatment of the HA specimens resulted in a significant bacterial reduction at a power output of 2.0 and 2.5 W only (Table 3).

The number of viable cells on specimens treated with 0.2% CHX was reduced by 2.96 to 3.96 log steps, corresponding to a 99.89% to 99.99% reduction.

### Heat Generation

As shown by the real-time video images (Figs 2a to 2d), irradiation induced an immediate, measurable temperature elevation on the implant surface. The heat generation was fastest during the initial phase of approximately 20 seconds. The highest temperatures were registered in the spot of irradiation. After 10 seconds, the temperature of the peri-implant bone was below the critical threshold of 47°C at power densities between 176.9 and 530.7 W/cm$^2$. Temperatures in the range of 88.1°C to 94.2°C were registered in the laser focus. At higher energy densities, the temperature on the adjacent bone surface exceeded the critical threshold within a time range of 11 to 20 seconds, and focus temperatures of over 134°C were registered. After 20 seconds, equilibrium between the energy supply and the cooling capacity of the system was set, resulting in only minor temperature rises thereafter. After a cooling period of 120 seconds, the initial temperatures were reached.

## DISCUSSION

The reductive effect of various laser systems on pathogenic oral bacteria has been demonstrated in vitro.[20–25] However, data obtained under standardized laboratory test conditions for the 809-nm diode laser have not been available. Moreover, the specific rough morphology of implant surfaces requires special consideration when evaluating the potential bactericidal effect of lasers. The results of this study indicated that the 809-nm semiconductor

**Table 1 Bacterial Counts in TPS Specimens**

| Group | Counts | | | | | Mean log kill | Bacterial reduction (%) | *P* |
|---|---|---|---|---|---|---|---|---|
| | Mean | Median | SD | Max | Min | | | |
| Control | 2.50E + 05 | 2.50E + 05 | 9.26E + 04 | 4.00E + 05 | 1.00E + 05 | | | |
| CHX | 2.69E + 01 | 2.50E + 01 | 1.53E + 01 | 5.00E + 01 | 1.00E + 01 | 3.97 | 99.99 | .00012 |
| 0.5 W | 1.38E + 05 | 1.00E + 05 | 5.18E + 04 | 2.00E + 05 | 1.00E + 05 | 0.26 | 45.00 | .02555 |
| 1.0 W | 3.00E + 04 | 2.50E + 04 | 1.69E + 04 | 6.00E + 04 | 2.00E + 04 | 0.92 | 88.00 | .00044 |
| 1.5 W | 1.75E + 03 | 1.50E + 03 | 1.04E + 03 | 4.00E + 03 | 1.00E + 03 | 2.15 | 99.30 | .00012 |
| 2.0 W | 1.50E + 03 | 1.00E + 03 | 7.56E + 02 | 3.00E + 03 | 1.00E + 03 | 2.22 | 99.40 | .00013 |
| 2.5 W | 2.25E + 03 | 2.00E + 03 | 1.67E + 03 | 6.00E + 03 | 1.00E + 03 | 2.05 | 99.10 | .00012 |

**Table 2 Bacterial Counts in SA Specimens**

| Group | Counts | | | | | Mean log kill | Bacterial reduction (%) | *P* |
|---|---|---|---|---|---|---|---|---|
| | Mean | Median | SD | Max | Min | | | |
| Control | 4.13E + 04 | 3.63E + 04 | 3.52E + 04 | 3.22E + 04 | 3.41E + 04 | | | |
| CHX | 4.50E + 01 | 4.35E + 01 | 4.54E + 01 | 4.46E + 01 | 4.15E + 01 | 2.96 | 99.89 | .00009 |
| 0.5 W | 1.75E + 04 | 1.50E + 04 | 8.86E + 03 | 3.00E + 04 | 1.00E + 04 | 0.37 | 57.58 | .00832 |
| 1.0 W | 1.63E + 04 | 1.50E + 04 | 7.44E + 03 | 3.00E + 04 | 1.00E + 04 | 0.40 | 60.61 | .00157 |
| 1.5 W | 2.75E + 03 | 2.50E + 03 | 1.49E + 03 | 5.00E + 03 | 1.00E + 03 | 1.18 | 93.33 | .00013 |
| 2.0 W | 3.01E + 03 | 2.50E + 03 | 1.84E + 03 | 5.00E + 03 | 4.30E + 01 | 1.14 | 92.71 | .00021 |
| 2.5 W | 1.63E + 01 | 1.50E = 01 | 7.44E + 00 | 3.00E + 01 | 1.00E + 01 | 3.40 | 99.96 | .00009 |

**Table 3 Bacterial Counts in HA Specimens**

| Group | Counts | | | | | Mean log kill | Bacterial reduction (%) | *P* |
|---|---|---|---|---|---|---|---|---|
| | Mean | Median | SD | Max | Min | | | |
| Control | 2.25E + 05 | 2.00E + 05 | 1.04E + 05 | 4.00E + 05 | 1.00E + 05 | | | |
| CHX | 3.25E + 01 | 3.00E + 01 | 1.28E + 01 | 5.00E + 01 | 2.00E + 01 | 3.84 | 99.99 | .00047 |
| 0.5 W | 1.88E + 05 | 2.00E + 05 | 8.35E + 04 | 3.00E + 05 | 1.00E + 05 | 0.08 | 16.67 | .35062 |
| 1.0 W | 1.75E + 05 | 2.00E + 05 | 7.07E + 04 | 3.00E + 05 | 1.00E + 05 | 0.11 | 22.22 | .43042 |
| 1.5 W | 1.50E + 05 | 1.50E + 05 | 5.35E + 04 | 2.00E + 05 | 1.00E + 05 | 0.18 | 33.33 | .07960 |
| 2.0 W | 4.00E + 03 | 4.00E + 03 | 1.31E + 03 | 6.00E + 03 | 2.00E + 03 | 1.75 | 98.22 | .00051 |
| 2.5 W | 1.63E + 03 | 1.50E + 03 | 7.44E + 02 | 3.00E + 03 | 1.00E + 03 | 2.14 | 99.28 | .00048 |

laser is capable of decontaminating rough implant surfaces. The differences in power densities necessary to decontaminate the respective surface imply that the surface structure and surface composition have an influence on the decontamination process. Monochromatic laser light of 809 nm is well absorbed by dark pigments and surfaces and highly reflected by bright surfaces, such as enamel or dentin. Hydroxyapatite-coated presumably has a considerably higher reflective capacity than the darker titanium surfaces. Therefore, a higher power density is necessary to achieve comparable thermal effects in the spot of irradiation. It can be assumed that surfaces treated with the laser were not sterilized, since even a temperature of 100°C was not exceeded in the spot of irradiation because the fiber was moved at a constant speed over the specimen. Thermographic analysis demonstrated that, in an energy-dependent manner, it takes 10 seconds or more of constant irradiation in one spot to exceed a temperature of 100°C on the titanium surface. Rather, the thermal effects on the laser surface resulted in temperature elevations that induced protein denaturation and led to cell necrosis. Direct absorption of laser light by the cells leading to evaporation might also be noted, although water (the main cell component) has only a moderate absorption capacity to light with a wavelength of 809 nm.

Surface characteristics also influenced the adhesion process. Comparable to the data presented, preliminary experiments revealed that the bacterial adherence of *S sanguinis*, a primary colonizer of enamel and implant surfaces that can lead to secondary adhesion of pathogenic bacteria,[26,27] was lower on the SA surface than on the TPS and HA surfaces.

***Figs 2a to 2d*** *Representative thermograms of a TPS stepped-cylinder implant irradiated at 1.5 W (continuous wave) in one spot.*

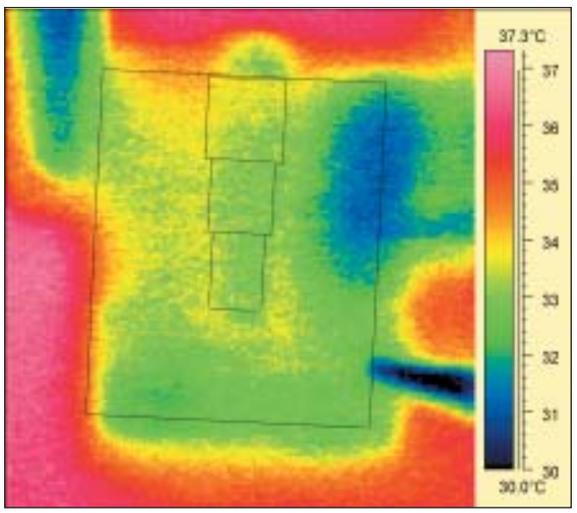


**Fig 2a** The bone block with the implant was placed into a water bath and stabilized at a temperature of 37°C prior to irradiation.

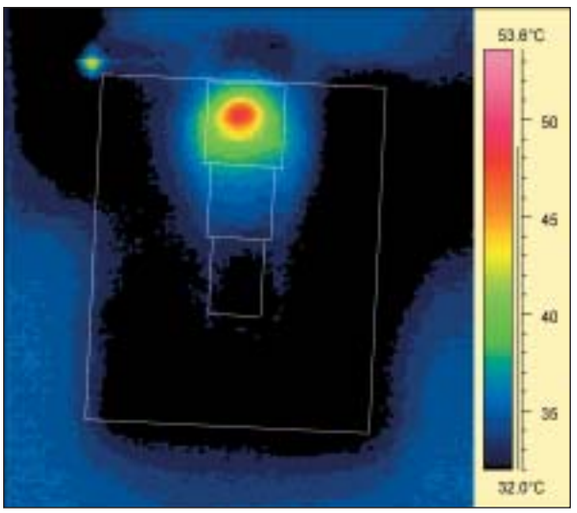


**Fig 2b** Irradiation induced an immediate temperature elevation on the implant surface.

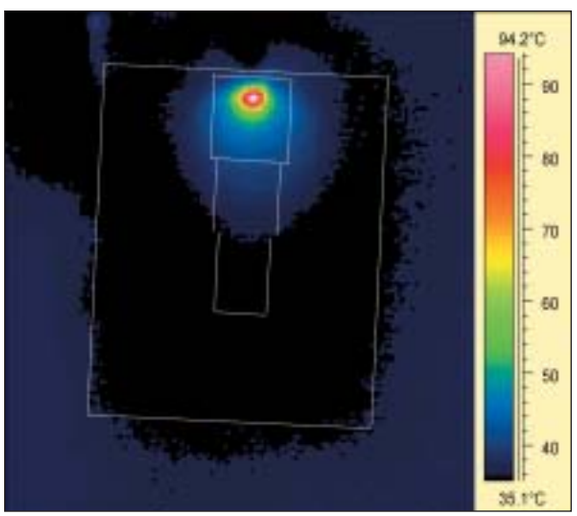


**Fig 2c** After 10 seconds, the temperature in the lased spot exceeded 100°C. Bone temperature was just below the critical threshold of 47°C.

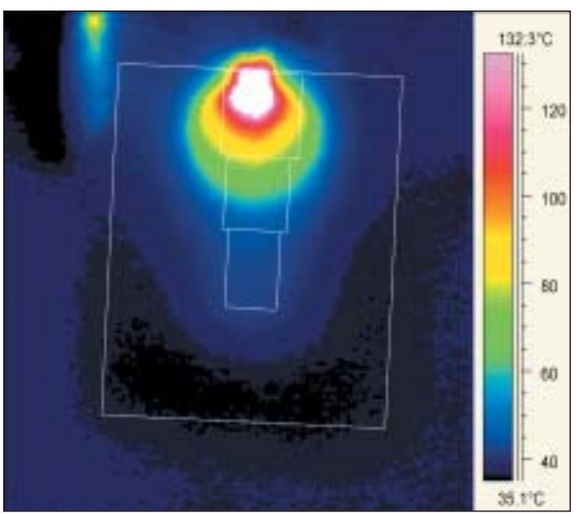


**Fig 2d** After 20 seconds, accumulation of excessive temperature elevations was measured in the adjacent bone.

Despite the considerable reduction in the number of bacteria resulting from laser irradiation, it should be noted that a superior bactericidal effect was achieved by a 1-minute treatment with CHX. In this in vitro study, however, only 1 bacterial species was investigated. The influence of a biofilm as formed in the oral cavity could not be simulated in vitro. It is known that within a biofilm, bacteria have an altered susceptibility to chemical agents. To the authors' knowledge, data on the influence of biofilm formation on the susceptibility of bacteria to laser light are not available. It could be assumed, however, to be negligible, since laser decontamination is a physical process. The power output of the laser system used in the present investigation was limited to 2.5 W, since bone vitality would be jeopardized at higher energy settings in an in vivo situation.

Deppe and coworkers demonstrated that application of the $CO_2$ laser in the treatment of ailing implants may favorably influence new osseous regeneration in beagle dogs.[9,14] It is not known whether this induction of bone formation is the result of the elimination of inflammation only or a result of stimulation of bone growth by laser light. This phenomenon requires further investigation with regard to different wavelengths in particular.

The potential ability of lasers to denature proteins might be useful in the elimination of endotoxins from implant surfaces. Therefore, further research is necessary to evaluate laser application as a useful tool in the treatment of peri-implant infection.

## CONCLUSIONS

Within the limitations of an in vitro study, it can be concluded that implant surface decontamination by means of the 809-nm diode laser is possible. The bactericidal effect, however, is less potent than that achieved by CHX. Application of this type of laser requires special consideration for excessive heat generation in the peri-implant bone.

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