

Laser antisepsis of *Porphyromonas gingivalis* in vitro with dental lasers.

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ABSTRACT

It has been shown that both pulsed Nd:YAG (1064nm) and continuous diode (810nm) dental lasers kill pathogenic bacteria (laser antisepsis), but a quantitative method for determining clinical dosimetry does not exist. The purpose of this study was to develop a method to quantify the efficacy of ablation of *Porphyromonas gingivalis* (*Pg*) *in vitro* for two different lasers. The ablation thresholds for the two lasers were compared in the following manner. The energy density was measured as a function of distance from the output of the fiber-optic delivery system. *Pg* cultures were grown on blood agar plates under standard anaerobic conditions. Blood agar provides an approximation of gingival tissue for the wavelengths tested in having hemoglobin as a primary absorber. Single pulses (Nd:YAG: 100- μ s; diode: 100-msec) of laser energy were delivered to *Pg* colonies and the energy density was increased until the appearance of a small plume was observed coincident with a laser pulse. The energy density at this point defines the ablation threshold. Ablation thresholds to a single pulse were determined for both *Pg* and for blood agar alone. The large difference in ablation thresholds between the pigmented pathogen and the host matrix for pulsed-Nd:YAG represented a significant therapeutic ratio and *Pg* was ablated without visible effect on the blood agar. Near threshold the 810-nm diode laser destroyed both the pathogen and the gel. Clinically, the pulsed Nd:YAG may selectively destroy pigmented pathogens leaving the surrounding tissue intact. The 810-nm diode laser may not demonstrate this selectivity due to its longer pulse length and greater absorption by hemoglobin.

Key words: Periodontitis, Diode and Nd:YAG dental laser, Bacterial reduction, Laser antisepsis, *P. gingivalis*.

1. INTRODUCTION

Porphyromonas gingivalis (*Pg*) has been established as a pathogenic bacteria involved in the periodontal disease process (Celesk, 1979, Lamont, 1998). *Pg* are darkly pigmented, gram-negative, motile, anaerobic bacteria known to cause tissue destruction. *Pg* persists in the biofilm on tooth surfaces, adheres to and enter epithelial cells. Intracellular bacteria can evade host immune effectors and antibiotics commonly used to treat infection. *Pg* are known to colonize stagnant niches in calculus and cementum, and have been identified migrating far as 1-mm into the dentinal tubules and lacunae. These "privileged sites" are accessible with lasers.

Both pulsed Nd:YAG (1064nm) and continuous diode (810nm) dental lasers are in current use for treatment of periodontitis. It has been shown that laser treatment kills pathogenic bacteria (laser antisepsis) (Ben Hatit, 1996; Chan and Chien, 1994; Cobb, 1992; Gutknect, 1997; Hardee, 1994; Klinke, 1997; Lin, 1992; Moritz, 1997, 1998, 2000; Ramskold, 1997; Rooney, 1994; Tseng, 1992; and Whitters, 1994), but a quantitative method for determining clinical dosimetry does not yet exist.

OBJECTIVES:

- Quantify laser ablation of *Pg in vitro* for cw-diode (810-nm) and pulsed-Nd:YAG (1064-nm) dental lasers.
- Evaluate the selectivity of *Pg* destruction using an *in vitro* tissue model.
- Define an efficacious clinical dosimetry for laser antisepsis.

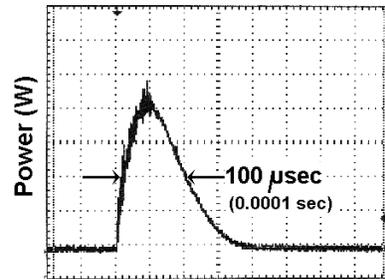
2. METHODOLOGY



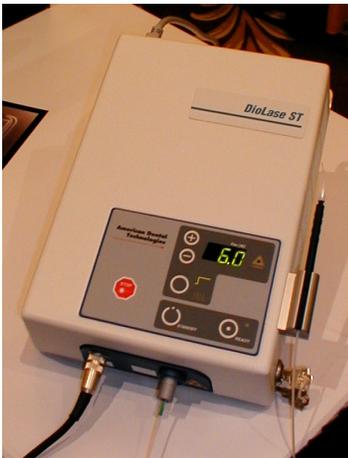
Pulsed Nd:YAG Dental Laser

Specifications:

Wavelength: 1064 nm
 Max average power: 6 Watts
 Mode: Pulsed
 Pulse repetition rate: 5-100 p/s (Hz)
 Pulse energy: 20-200 mJ
 Pulse width: 100 microsec
 Delivery: Optical fiber
 Aiming beam: 1 mW Red diode



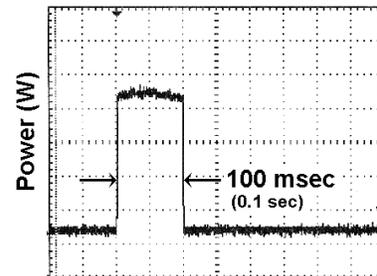
Single 100 microsecond, 100 mJ pulses were delivered in these experiments.



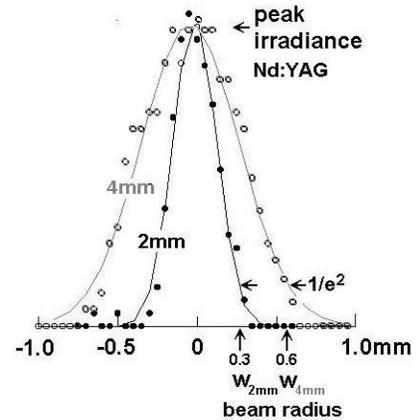
Continuous-Wave Diode Dental Laser

Specifications:

Wavelength: 810 nm
 Max power: 6 Watts
 Mode: Continuous
 Delivery: Optical fiber
 Aiming beam: 1 mW Red diode



Single pulses were generated by replacing the footswitch with a timing circuit. Single 100-msec pulses were delivered in these experiments.



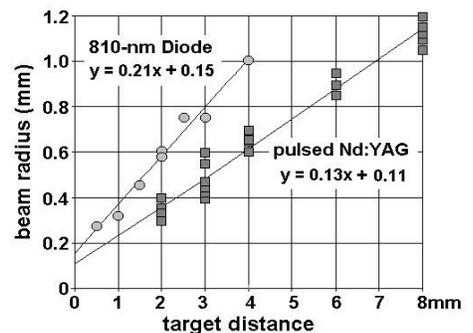
Bacteria And Blood Agar

Cultures of *P. gingivalis* 33277 were grown on rabbit blood agar. Colonies form a 30-60 micron thick biofilm on the agar surface. The blood agar approximates the optical properties of soft tissue (water, hemoglobin and other organics).

Laser Beam Calibration

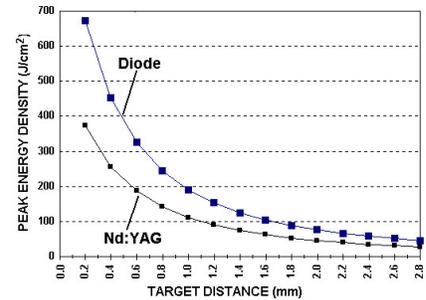
The laser beam is characterized by collecting beam profiles at various distances from the tip. The beam is directed into the energy meter and a straight edge is stepped across the beam. Profiles are constructed by measuring the change in energy between steps. Gaussian profiles of the Nd:YAG beam at 2-mm and 4-mm from the tip (320- μ m fiber) are shown to the right. From these functions we estimate the beam radius as the x value where $y = 1/e^2$ of the peak radiant energy.

Beam radii are plotted in the lower right for both the 810-nm diode and the 1064-nm pulsed Nd:YAG. The least squares best-fit regression estimates the divergence of the beam and provides a computational formula for determining the beam radius at any distance from the fiber tip.



The average radiant energy at any target distance is equal the total energy in the beam (measured with the energy meter) divided by the spot area (πr^2) at that distance.. It is necessary to specify ablation thresholds with the peak radiant energy because ablation occurs first in the center of the beam where the energy density is greatest. For a Gaussian beam the peak is exactly equal to two times the average.

Peak radiant energy as a function of target distance can be computed from these measurements. Shown here is the function for a 93-mJ pulse from the Nd:YAG and a 389-mJ pulse from the diode.

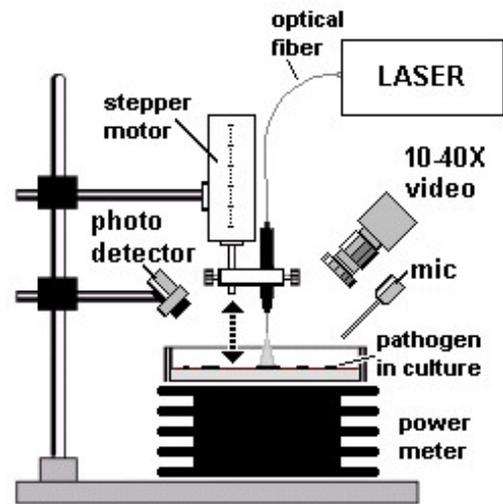


Bioassay

The absorption of laser energy induces a change of state of the target from the solid or liquid state to the gas or vapor state. Laser ablation is the removal or destruction of tissue by radiant exposure incident on the tissue surface. Laser ablation of *Pg* is a quantifiable photophysical event related to bacterial reduction.

A basic physical characterization of tissue ablation by laser energy is that as radiant exposure increases, the depth of tissue removal increases. Very low level exposure has no effect and there is a threshold energy dose necessary to ablate a just measurable amount of tissue. For laser antiseptics the threshold radiant energy that is lethal to the target pathogen is defined by this minimum, the ablation threshold. This is significant since ablation threshold defines a radiant exposure that is toxic to the target pathogen at or above this value.

We use the bioassay set-up shown to the right to determine the sensitivity of bacteria in culture to laser irradiation. Specimens are placed in the beam path. The fiber tip is lowered to the culture surface and the stepper motor readout (2- μ m steps) is set to zero. Fiber tip distances above the culture surface are converted to peak radiant energy using the calibration described above. Target tissue is irradiated with single pulses under direct observation with 40X magnification.

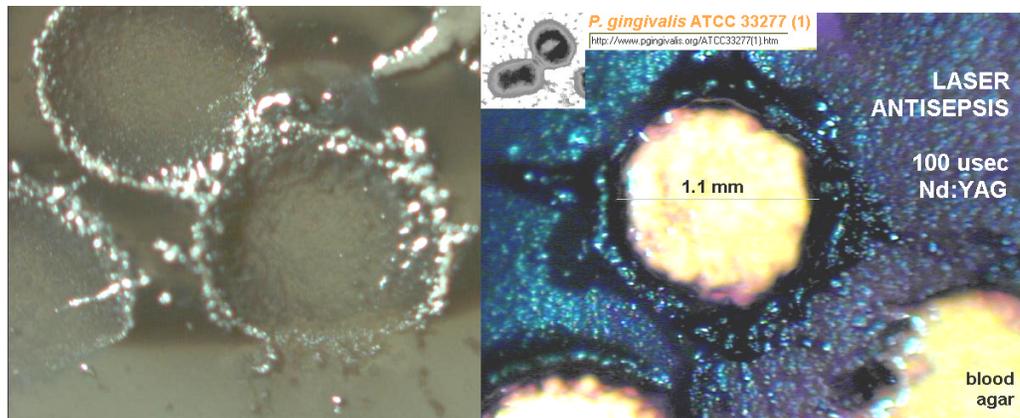


3. RESULTS AND SIGNIFICANCE

Ablation Threshold

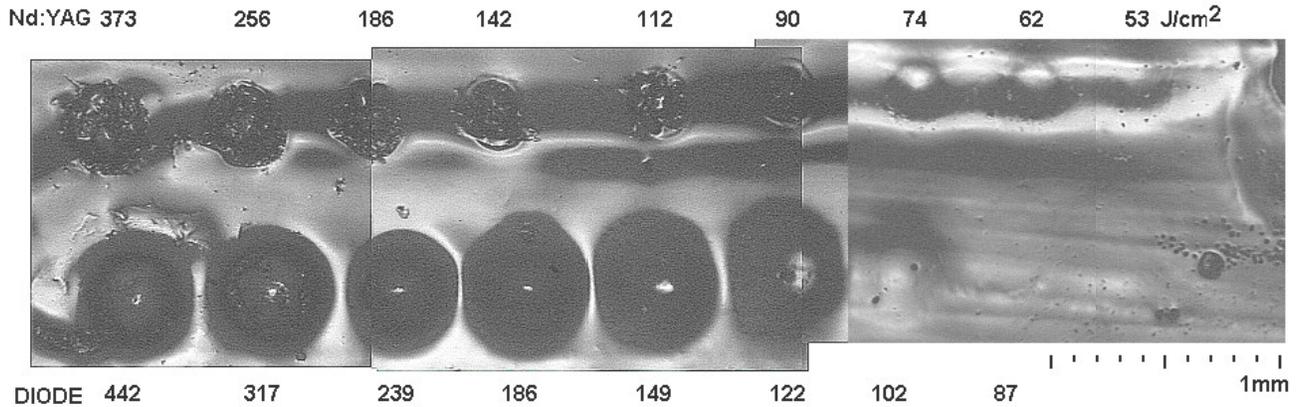
We use a visual detection method to identify ablation thresholds. At high radiant energy each pulse creates an obvious crater. At lower radiant energies blanching appears in the center of the beam on the target surface. As the energy is lowered the blanched area observed decreases in size, indicating less material removed. About 5-J/cm² below the threshold of the “surface blanching effect” a small plume of ablated material is emitted from the target surface. At lower energies there is no visible effect. The radiant energy level between these last two observations defines ablation threshold.

Single 217 mJ/cm² peak radiant energy pulses from the Nd:YAG vaporize all bacteria from within the laser spot, but leave the substrate blood agar intact.



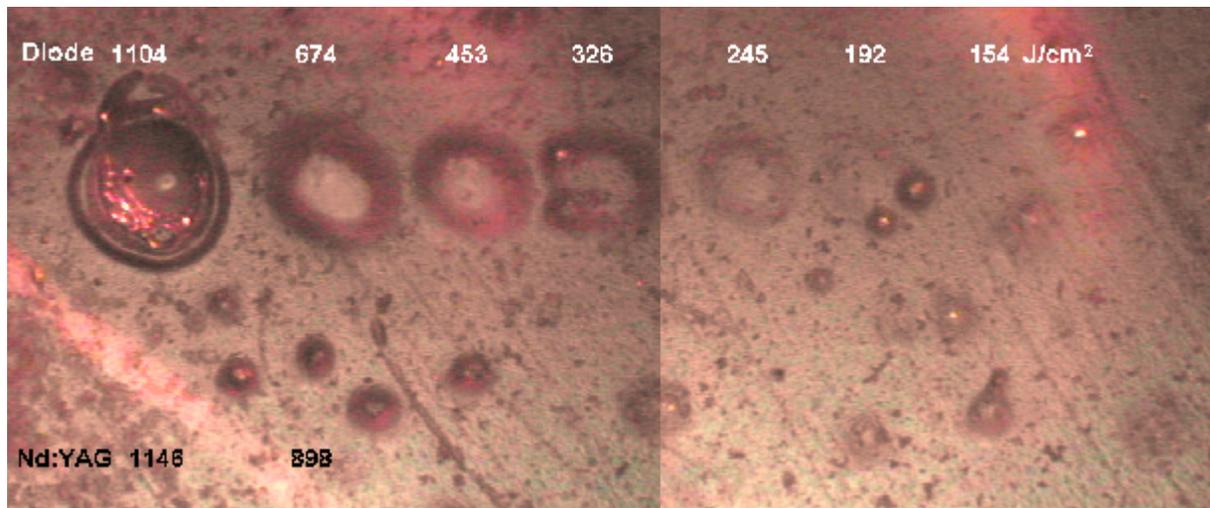
Notice the Nd:YAG craters have a flat bottom indicating that no agar was ablated.

Pg Ablation Thresholds: Nd:YAG and Diode



Shown above are two series of impact craters where the distance between the culture surface and the fiber tip was increased in 100-micron steps from left to right and single laser pulses delivered. Threshold determined for the Nd:YAG was 50 J/cm² and for the diode, 95 J/cm². Notice that the diode craters do not have a flat bottom.

Blood Agar Ablation Thresholds: Nd:YAG and Diode



This figure shows two series on blood agar alone. Threshold determined for the diode was 173 J/cm². The Nd:YAG had no visible effect at the highest peak energy density available. Slight dehydration of the agar reveals the thermal effects in more detail.

Ablation Thresholds Comparison

Ablation thresholds: peak energy density (J/cm²). Average of 12 measurements for Nd:YAG and 9 for diode.

	Diode	Nd:YAG
<i>P_g</i>	96	58
Agar	146	>1400
Ther. Index	1.5	> 24

Therapeutic Index

The therapeutic index is defined by the ratio of the laser energy dose that destroys pathogens (therapeutic dose) to the energy dose that damages normal tissue (toxic dose).

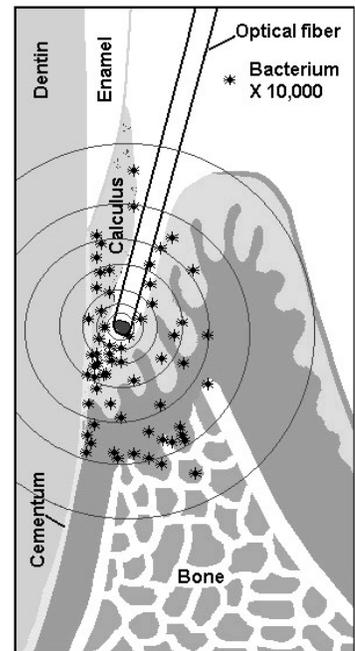
- Therapeutic dose: A) Level that destroys pathogens
- Toxic dose: B) Level that destroys normal tissue

- Therapeutic index: B/A

For these experiments the therapeutic indices using our *Pg* biofilm/soft tissue model are:

Diode 146 / 96 (J/cm²) = **1.5**
 Nd:YAG >1400 / 58 = **>24**

The difference in therapeutic index between Nd:YAG and diode indicates that the pulsed Nd:YAG has a 16 times greater selectivity for destruction of pigmented oral pathogens than the diode laser.



Depth of Kill

Infrared light penetrates deep into tissue. If surface radiant energy is maintained below the surface damage threshold (toxic dose) there remains a volume of tissue below the surface wherein energy deposition is lethal to pigmented bacteria. The greater the therapeutic index, the greater the potential depth of antiseptis.

Laser antiseptis vs. chemical antibiotics.

	<u>DRUGS</u>	<u>LASER</u>
Side effects	systemic	none
Resistance	yes	No (albino mutant?)
Negative interactions	possible	none
Spectrum of activity	broad or narrow	pigmented pathogens
Local delivery mode	chemical dissolution	light diffusion

4. CLINICAL RELEVANCE

When used for sulcular debridement and other dental treatments, the 100-isec pulsed Nd:YAG may selectively destroy pigmented pathogens to a depth below the tissue surface leaving the surrounding tissue intact.

5. ACKNOWLEDGMENTS

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