

SHORT COMMUNICATION

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The bactericidal effect of a Genius[®] Nd:YAG laser

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Abstract: *Purpose:* To evaluate the 'in vitro' bactericidal effect of the Nd:YAG laser (Genius, MØlsgaard Dental, Copenhagen, Denmark) on six periodontal pathogens. *Methods:* Suspensions of six different periodontal pathogens (*Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, *Fusobacterium nucleatum* and *Parvimonas micra*) were prepared in small Eppendorff tubes, and exposed to a Nd:YAG laser for five different periods of time. Laser settings used: Power 6 Watt (on a scale of 1–12 W), Frequency 50 Hz, Pulse duration 250 µs. After exposure to the laser, aliquots of the suspensions were spread on blood agar plates for bacterial counting. *Results:* After 5 s of laser exposure, there was a decrease in total colony forming units for all six selected microorganisms. After 15, 30 and 45 s, no viable bacterial cells could be retrieved. *Conclusion:* In this 'in vitro' model, 15 s of Nd:YAG laser use was found to be effective for total killing of the six tested periodontal pathogens.

Key words: *in vitro*; laser; microorganisms; Nd:YAG

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Introduction

Most treatment modalities used in periodontal therapy aim to reduce the bacterial plaque on the root surface to levels compatible with periodontal health. The effectiveness of scaling and root planing in the treatment of periodontal disease is universally accepted (1, 2). Some studies suggest that the outcome of periodontal treatment is better if particular suspected pathogens can no longer be detected after therapy and that sites which remain positive are at greater risk of further breakdown (3). Laser light has several unique properties that make it useful for

a variety of applications in dental care. It is capable of ablating and vapourizing residual organic debris, including microbial plaque, and has the potential to disinfect and remove pocket sulcular lining (4–6). Additional therapy such as laser energy aims at reducing or eliminating bacteria and might therefore be clinically useful to reduce soft tissue inflammation. A number of authors have demonstrated the bactericidal effect of laser energy (4, 7–11). Absorption of laser light is one of the most important factors in laser tissue interaction. The high power Nd:YAG laser, which contains a crystal of neodymium-doped yttrium aluminium garnet as the active medium, produces light at 1064 nm in the near infrared spectrum. This laser emitting light is well absorbed by melanin and haemoglobin. The energy is transmitted through water and poorly absorbed in hydroxyapatite. This makes it an excellent choice to use in a periodontally inflamed pocket which is well vascularized studied by Raffetto (17). The laser energy is transmitted with a thin, flexible, fibre optic system in a small hand piece that allows the clinician to access the subgingival area. Given the characteristics of laser light, direct contact between the target and the fibre tip is not required suggested by Bergmans *et al.* (18). Among dentists and dental hygienists in the Netherlands, the water cooled pulsed Nd:Yag laser is used as an adjunct to the ‘non-surgical’ treatment of periodontitis, as has been suggested by Lioubavina *et al.* (6).

The purpose of this study was to investigate the bactericidal effect of the Genius Nd:YAG laser (Genius Periodontal A/S, Mølsgaard Dental, Copenhagen, Denmark) by determining the time necessary to kill periodontal pathogens ‘*in vitro*’.

Material and methods

Laser

A solid state crystal Nd:YAG laser (Genius Periodontal A/S) was used at the settings, as shown in Table 1. The Genius Nd:YAG laser with water and air coolant has an optical fibre tip, approximately the size of a periodontal probe (0.6 mm in diameter).

Table 1. Nd:YAG laser settings

Power	6	1–12 W
Water	0	1–12 W
Air	0	1–12 W
Frequency	50	10–100 Hz
Pulse duration	250	100–800 μ s
Pulse energy	400	400–800 mJ

In vitro procedures

Small Eppendorff tubes were used in an ‘*in vitro*’ experiment to study the microbiological effects of laser treatment on the killing of periodontal pathogens. Fresh clinical isolates of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, *Fusobacterium nucleatum* and *Parvimonas micra* were retrieved from subgingival plaque samples of patients with chronic periodontitis. Subgingival plaque samples were grown on 5% horse blood agar plates supplemented with haemin (5 mg l⁻¹) and menadione (1 mg l⁻¹) and incubated for 14 days at 37°C in 80% N₂, 10% CO₂ and 10% H₂ (13). Identification was based on Gram stain, cell and colony morphology, air tolerance, production of catalase and on a number of biochemical reactions. *Aggregatibacter actinomycetemcomitans* was isolated on tryptic soy-serum-bicitracin-vancomycin (TSBV) plates incubated in air with 5% CO₂ at 37°C for 3 days (14). Isolates were identified on the basis of characteristic colony morphology (star-like inner structure) on TSBV, a positive catalase reaction with 3% hydrogen peroxide and a set of specific enzymes (APIZym; La Balme les Grottes, Montalieu-Vercieu, France).

Bacterial strains were sub-cultured on blood agar base for 72 h and suspensions were prepared in phosphate-buffered saline of approximately 10⁵ colony forming units per ml. The test volume was 40 μ l in an Eppendorff PCR-vial. Before starting the experiment, 20 μ l was extracted and spread on a culture plate for a baseline assessment. Measurements were performed in triplicate for each single microorganism and exposure time. Before use, the laser was activated on a piece of carbon paper. Bacterial suspensions were prepared to be exposed to a Nd:YAG laser for four different periods of time:



Fig. 1. Laser tip (on the left) was introduced into the Eppendorff tube (on the right) which was followed by microbial plating.

Table 2. Presence (+) or absence (–) of cultivable microbiological species on the culture plates related to the different exposure time of the Nd:YAG laser in seconds

Exposure time (s)	Aa	Pg	Pi	Tf	Pm	Fn
Baseline	+	+	+	+	+	+
0	+	+	+	+	+	+
5	+	+	+	+	+	+
15	–	–	–	–	–	–
30	–	–	–	–	–	–
45	–	–	–	–	–	–

Aa, *Aggregatibacter actinomycetemcomitans*; Pg, *Porphyromonas gingivalis*; Pi, *Prevotella intermedia*; Tf, *Tannerella forsythia*; Pm, *Parvimonas micra*; Fn, *Fusobacterium nucleatum*.

5, 15, 30 and 45 s. The tip of the laser fibre was decontaminated with alcohol before use. The laser tip was placed in the Eppendorff tube and small horizontal excursions were made in the direction of the bottom during the four different exposure times. Laser settings were: Power 6 W as suggested by the manufacturer (on a scale of 1–12 W), Frequency 50 Hz, Pulse duration 250 μ s. The water and air coolant, typical for the Genius® laser, was not activated during this procedure to avoid dilution of the dissolved microorganisms. To compensate for the lack of water and air coolant, Eppendorff tubes were kept on ice during the experiment. After exposure to the laser, the solution was extracted from the tubes and microbial plating was performed (Fig. 1). Solutions were plated on blood agar plates to test for survival of the specific periodontal pathogens. For negative controls, the same procedure was performed without activating the laser (0 s).

Results

Table 2 shows the presence or absence of bacterial growth of specific microbiological species on the culture plates in relation to exposure time of the Nd:YAG laser. As a control treatment, the baseline as well as exposure to a non-activated Nd:YAG laser (0 s) showed confluent growth on all culture plates of the test bacteria (Fig. 2a and b). After 5 s of exposure, a decrease in total colony forming units of all test species was noted (Fig. 2c). After exposure of 15 s, no colony forming units were observed for any of the six selected microbiological species. Consequently at 30 and 45 s of exposure, for all the six periodontal pathogens, the culture plates were sterile (Fig. 2d).

Discussion

The most frequently used method to reduce the numbers of putative periodontal pathogens from the periodontal pocket is mechanical debridement of the root surfaces. This can be car-

ried out through non-surgical scaling and root planing, or by surgical procedures aimed at the elimination of deepened pockets. Furthermore, antibiotics and antiseptics are used as an adjunctive to mechanical debridement to obtain elimination of certain bacterial species.

More recently, it has been suggested that high-power lasers, such as the Nd:YAG laser, may be useful for destroying bacteria, presumably by a thermal effect (9, 17, 18). The bactericidal action of a high-power Nd:YAG laser on a suspension of *Escherichia coli* was studied. In a thermocouple equipped cuvette, a temperature rise up to 50°C was observed after the use of laser with a power output of 100 W for 23 s. This resulted in more than 90% loss of viability of *E. coli*. As a comparison, a suspension of *E. coli* was heated up to 50°C in a water bath. In this case, there was only a minimal loss of viability of *E. coli*. It was concluded that ‘thermal’ response because of just heating the suspension fluid is not a sufficient explanation for the killing of *E. coli*. Therefore, other processes may be involved during exposure to the Nd:YAG laser (18). However, using a different ‘*in vitro*’ model in which agar plates (inoculated with bacteria) were exposed to laser light, temperatures close to 100°C were measured immediately after radiation. This indicates that the antimicrobial effect within this particular model is probably caused by a photo-thermal effect rather than by photo-chemical effects of the laser light itself as suggested by Grönquist *et al.* (20). Meral *et al.* (19) evaluated the minimal energy levels necessary to have a bactericidal effect on a bacterial suspension of various oral microorganisms. Instead of submerging the tip in the suspension, they observed a distance of 10 mm. Within this laboratory setting, they concluded that the addition of a dye (e.g. sheep blood) enhanced the absorption of the laser light.

The efficacy of the Nd:YAG lasers has also been evaluated for the photo-thermal disinfection of root canals studied by Bergmans *et al.* (21). Although a different indication, the ‘*in vitro*’ models used bare resemblance to the present investigation. Laser irradiation was performed in wet root canals that had been inoculated with a bacterial suspension of *Enterococcus faecalis* (a relatively heat-resistant bacterium). The laser application was found to be safe and to have a potential disinfection capacity (99.7% kill). In a comparable study by Wang *et al.* (21), the Er,Cr:YSGG laser was compared with the Nd:YAG laser. Both laser systems had a significant bactericidal effect on infected root canals, but the Nd:YAG was more effective resulting in 98% kill. In a more basic ‘*in vitro*’ endodontic model, laser radiation was delivered to 12 μ l *E. faecalis* broth cultures in small capillary tubes. For specimens that received a total energy of ≥ 60 J, no viable bacteria were detected following laser treatment (Rooney *et al.* 1994).

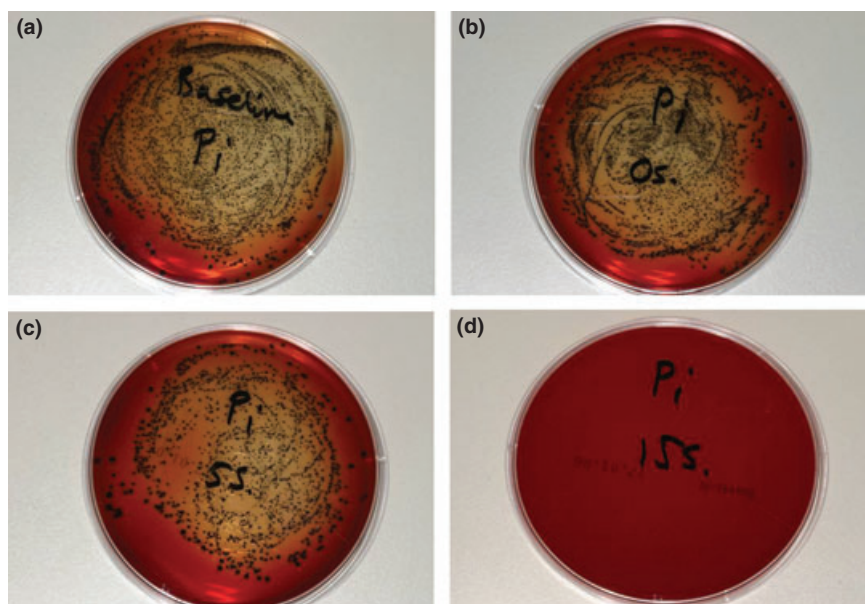


Fig. 2. (a) Total colony forming units of *Prevotella intermedia* at baseline; (b) 0 s of exposure; (c) 5 s of exposure; (d) 15 s of exposure.

In the present experiment, energy settings were used at a fixed level as a clear dose response between percentage of bacteria killed and energy density used is shown in the literature (7–9). Differences in contact time might explain the effectiveness of eliminating periodontal pathogens as the degree of vapourization, and thereby the possible elimination of periodontal pathogens takes place in the Eppendorff tubes. This elimination of pathogens is proportional to the amount of energy absorbed by the water. Energy is a product of power and duration of exposure (contact time); therefore, it is possible to alter the depth of penetration by changing either the lasers' power or the duration of the exposure, thereby increasing the bactericidal effect. Indeed, the present experiment revealed the usefulness of the Nd:YAG laser in eliminating periodontal pathogens as a function of exposure time.

As contemporary periodontal treatment aims at a general reduction of supra and subgingival bacterial mass by scaling and planing the root surfaces, the Nd:YAG laser might be a useful adjunct to this treatment in eliminating bacteria.

Conclusion

In this 'in vitro' model, 15 s of Nd:YAG laser use was found to be effective for total killing of the six tested periodontal pathogens.

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