# Bactericidal Effect of Nd:YAG Laser in an In Vitro Tissue Model – A Light Microscopic Evaluation

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**Purpose:** To evaluate the efficacy of Nd:YAG laser in destruction of Porphyromonas gingivalis and Prevotella intermedia and to assess surface damage after ablation with laser in an in vitro tissue model.

**Materials and Methods:** Subgingival plaque samples were collected from patients with generalized chronic periodontitis with a sterile curette. P. gingivalis and P. intermedia were isolated and cultured on blood agar plate which was the test group, while blood agar plate without colonies was the control group. Nd:YAG laser was applied to both plates and the results were observed under a surgical microscope at 24X magnification.

**Results:** Distinct craters with flat bottoms were seen in the test group after application of Nd:YAG laser, due to lysis of the colony forming units. No change was noted on the surface of the control group after irradiation with Nd:YAG laser.

**Conclusion:** Nd:YAG laser can selectively destroy pigmented periopathogenic bacteria, while leaving the surrounding tissue intact.

**Keywords:** Nd:YAG laser, Porphyromonas gingivalis, ablation, blood agar.

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Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by specific micro-organisms. Porphyromonas gingivalis and Prevotella intermedia are the predominant pathogenic bacteria involved in the periodontal disease process. They are darkly pigmented, gram-negative, non-motile, anaerobic rods. They are tissue invasive and have also been identified migrating up to the dentinal tubules.[1] Nonsurgical therapy remains the gold standard for periodontal therapy. However, since periodontal pathogens are capable of penetrating the tissues, nonsurgical therapy alone may not be adequate to eliminate the pathogenic bacteria.

Laser antisepsis is being extensively used as an adjunct to routine periodontal therapy in order to ensure elimination of pathogenic bacteria. Different laser systems with different wavelengths and properties are used in periodontal therapy.[2] Nd:YAG laser beam can penetrate up to 2 ± 1 mm into the tissue[3] and up to 1 mm into dentin,[4] thus affecting the tissue-invasive bacteria. P. gingivalis and P. intermedia are known to colonize stagnant niches in calculus and cementum which are inaccessible by nonsurgical periodontal therapy.

Neodymium:yttrium-aluminum-garnet (Nd:YAG) laser is a free-running pulsed-wave laser with a wavelength of 1064 nm. Several studies have suggested

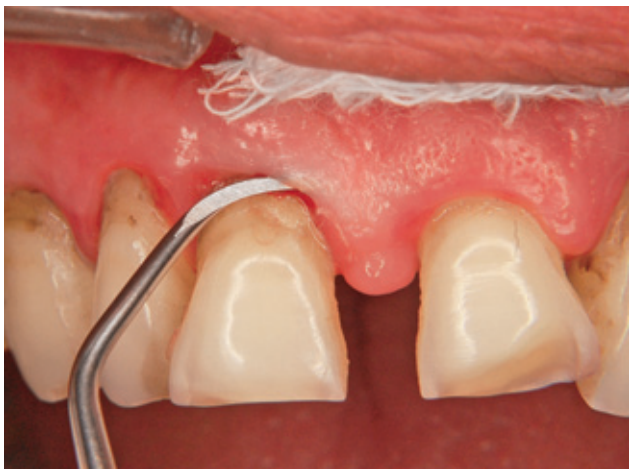

**Fig 1** Collection of subgingival plaque sample with a sterile curette.

reduction of probing depth, gain in clinical attachment level, and decreased microbiological count[5-8] as well as regeneration of periodontal structures[9] when used at appropriate settings. The photothermal effect of the Nd:YAG laser is useful for soft tissue surgery. Ablation (removal of tissue) occurs when photons are absorbed by the target and the resulting temperature increase is sufficient to vaporize or thermally coagulate tissue within the laser beam.

Thus, the purpose of the study was to evaluate the efficacy of Nd:YAG laser in destruction of Porphyromonas gingivalis and Prevotella intermedia and to assess surface damage after laser irradiation in an in vitro tissue model.

## MATERIALS AND METHODS

The study was done in the Department of Periodontology and Implantology, M.A.Rangoonwala College of Dental Sciences and Research Centre, Pune, India.

Inclusion criteria for collection of subgingival plaque samples:

- Both male and female patients within the age range of 30 to 45 years.
- Patients with generalized chronic periodontitis (according to the criteria of the World Workshop in Periodontics 1999).

Pregnant women, patients with any systemic disease, habit of smoking or tobacco, or history of administration of antibiotics in the past three months were excluded from the study.

Subgingival plaque samples were collected from two patients with generalized chronic periodontitis with the help of a sterile curette. The area was isolated with sterile gauze and dried to avoid contamination with saliva. Supragingival plaque and calculus were removed with a sterile curette to ensure the collection of subgingival microbial flora in the plaque sample (Fig 1).

The samples were transported to the microbiology lab (Jehangir Hospital, Pune) in a reduced thioglycollate broth within 1 h of collection. The samples were plated on sheep blood agar and incubated in an anaerobic jar for 48 to 72 h. Sheep blood agar was enriched with 0.0005% hemin and 0.00005% menadione. Colonies were subcultured on chocolate agar and incubated in a carbon dioxide jar. Obligate anaerobes do not grow on chocolate agar. Thus they were separated from facultative anaerobes. Pure cultures could be obtained from one of the samples.

Colonies of Porphyromonas gingivalis and Prevotella intermedia were identified on the basis of pigmentation, colony morphology, motility, and catalase and indole activity.

The test group consisted of blood agar plate with cultures of P. gingivalis and P. intermedia and the control group consisted of plain blood agar without cultures (Figs 2 and 3).

Irradiation of both test and control groups was done using Nd:YAG laser (Fotona) at the following parameters:[10]

- Power: 1.5 W
- Frequency: 15 Hz
- Duration: 5 s x 3 cycles
- Mode: short pulse
- Delivery system: optical fiber (300 µm)

The lids of the plates were opened just before irradiation, thus preventing previous exposure to oxygen. Irradiation of colony forming units (test group) and surface of blood agar (control group) was done in a noncontact mode from a distance of 2 mm.

Results were observed with a surgical microscope (Moller Wedel, Germany) under 24X magnification.

## RESULTS

Black pigmented colonies of P. gingivalis and P. intermedia in the test group were observed under the surgical

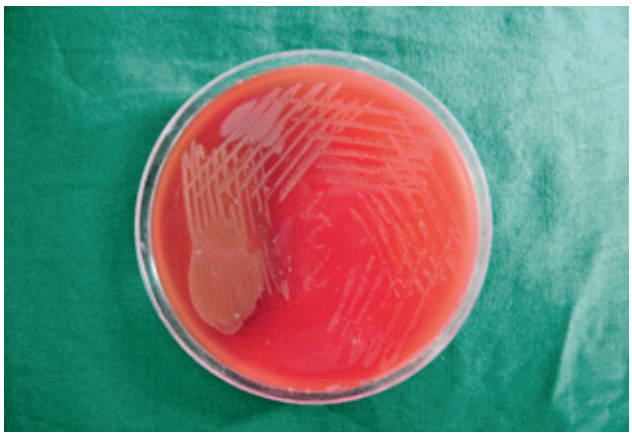

**Fig 2** Test group cultures of P. gingivalis and P. intermedia on blood agar plate.

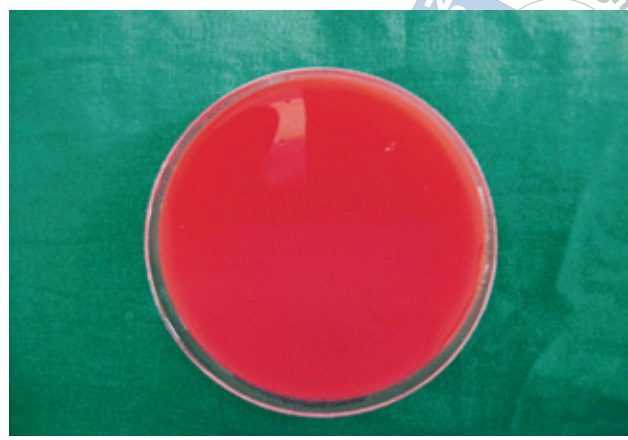

**Fig 3** Control group: blood agar plate without any microbial colonies.

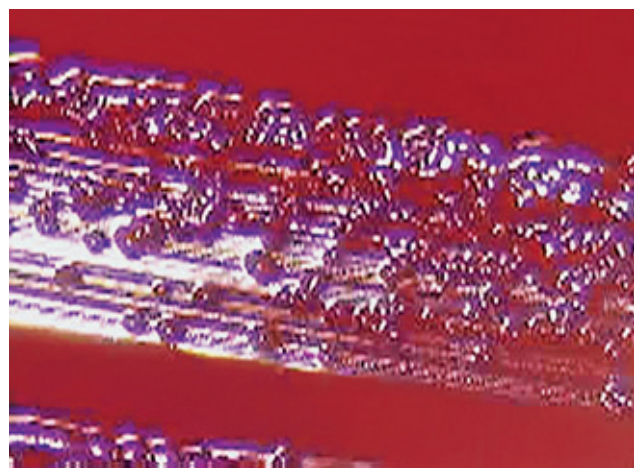

**Fig 4** Black pigmented colonies of P. gingivalis and P. intermedia seen under the surgical microscope at 24X magnification (test group).

microscope (24X magnification) before irradiation (Fig 4). The surface of the blood agar plate of the control group looked clear and did not show any evidence of microbial growth when observed under the microscope before irradiation (Fig 5).

After irradiation, the treated areas in both the test and control group were observed under 24X magnification. Distinct craters with flat bottoms were seen in the areas of ablation in the test group due to lysis of the colony forming units of the micro-organisms (Fig 6). No change was noted on the surface of the blood agar of the control group after ablation with Nd:YAG laser (Fig 7).

## DISCUSSION

P. gingivalis and P. intermedia are gram negative, anaerobic, motile bacteria which play a pivotal role in the pathogenesis, progression, and recurrence of periodontal disease. They persist in the mixed-species plaque biofilm on tooth surfaces, adhere to and enter the epithelial cells,[11] colonize calculus and cementum, and can penetrate dentinal tubules up to 1 mm.[12] These are sources for recolonization and these sites are not always accessible with the conventional treatment modalities. Eradication or adequate suppression of these pathogenic bacteria is therefore required for successful periodontal therapy.

**Fig 5** Surface of the blood agar plate without microbial colonies seen under the surgical microscope at 24X magnification (control group).

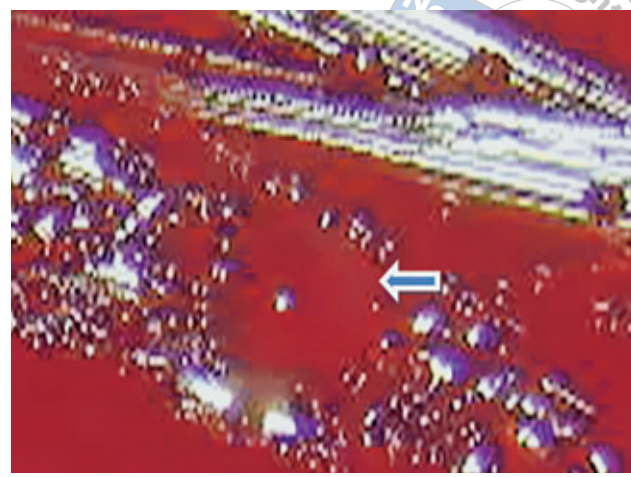

**Fig 6** Distinct flat-bottomed craters seen in the areas of laser irradiation due to lysis of colony forming units as seen under surgical microscope at 24X magnification.

**Fig 7** No change was noted on the surface of the blood agar after laser irradiation when seen under the surgical microscope at 24X magnification.

According to Schenk et al, sonic and ultrasonic instrumentation does not lead to killing of periopathogens.[13] The instrumentation helps to reduce the bacterial load by mechanical removal of plaque and calculus. The limitations of conventional therapy prompted us to implement the use of adjunctive anti-microbial measures.[14] Laser therapy has attracted attention due to its potential advantages. Nd:YAG laser beam can be delivered to a depth of 2 ± 1mm into the soft tissue.

Blood agar contains water, hemoglobin and other organics; hence, it approximates the optical properties of soft tissue. Distinct craters with flat bottoms were seen in the test group because of destruction of colony forming units of P. gingivalis and P. intermedia. The bottom of the craters did not indicate penetration of blood agar. The Nd:YAG laser wavelength of 1064 nm is better absorbed by the protohemin and protoporphyrin IX pigments of P. gingivalis and P. intermedia than by the hemoglobin or water in the blood agar. The absorption of Nd:YAG laser wavelength by the pigments in the bacteria leads to vaporization of water and cell lysis. This forms the basis of the selective bactericidal effect of Nd:YAG laser. Blood agar alone is therefore not affected when irradiated at the same settings. This study proves the selective ablation capacity of the Nd:YAG laser.

The selectivity of a drug is the concentration that destroys pathogens but is benign to normal tissue. It is expressed as the ratio of the drug concentration (mg/kg) that is toxic to normal tissue (toxic dose) to the drug dose that is toxic to the target pathogen (therapeutic dose). This is referred to as the therapeutic

ratio or therapeutic index. In this case, the therapeutic index is the ratio of the toxic dose, which is the fluence that damages the normal tissues, and therapeutic dose, which is the minimum laser fluence that destroys pathogens.

There is a therapeutic and diagnostic window in biological tissues where light absorption is minimal and light transmission is greatest. This window is in the red to near-infrared region, approximately 800 to 1200 nm, with maximum transmission for wavelengths around 1064 nm.[15] At these wavelengths, a volume of tissue remains below the surface where the energy is deposited and is lethal to pigmented bacteria. A greater therapeutic index means a greater potential depth of antisepsis. Harris et al[16] determined and compared the ablation thresholds of diode (810 nm) and Nd:YAG laser on cultures of P. gingivalis on blood agar with a visual detection method. A power meter was used to determine the energy output. The energy density was increased till plume or crater formation was seen, and this determined the ablation threshold. Similarly, as the energy density was increased, toxic effects were noted on the blood agar. The therapeutic index for pulsed Nd:YAG was > 24 compared to that of diode laser, which was 1.5. Thus, the surface radiant exposure can be maintained below the toxic dose with the use of Nd:YAG laser.[16] A high value for the index also indicates high selectivity.

The present study has proved the bactericidal effect of Nd:YAG laser without any potential damage to the tissue surface. However, studies have reported that Nd:YAG laser irradiation produces unfavorable thermal changes on the root surface,[17-19] if used at higher power settings. However, variations in the manner and conditions of irradiation, such as energy output, use or nonuse of water irrigation, and the degree of contact tip angulation to the root surface, may produce different results. Nd:YAG laser can be used for curettage of periodontal pockets to remove the infected granulation tissue and epithelial lining as well as disinfection and detoxification of periodontal pockets, at relatively low power settings.[20] Tissue penetration of a diode laser is less than that of the Nd:YAG laser, while the rate of heat generation is higher, resulting in deeper coagulation and more charring on the surface compared to the Nd:YAG laser.[21] However, the therapeutic index of diode laser (810 nm) is 1.5, which is much less than Nd:YAG, and therefore exhibits less selectivity towards pigmented periopathogens.

Recently, use of Nd:YAG laser has been advocated in LANAP (Laser-assisted New Attachment Procedure), where new connective tissue attachment is seen following removal of soft tissue lining with Nd:YAG laser. Ishikawa et al[22] stated that Nd:YAG laser holds a promising future when used as an adjunct to conventional mechanical nonsurgical therapy.

## CONCLUSION

Nd:YAG laser has a high therapeutic index and can selectively destroy the pigmented periopathogenic bacteria, while leaving the surrounding tissue intact. Nd:YAG laser has a favorable wavelength and convenient delivery system which makes its easy to use in the periodontal pockets. The selective bactericidal effect of lasers can be of great help in controlling the microbial etiology of periodontal disease, which is otherwise a challenge, as the oral cavity harbors more than 500 species of microorganisms.

Further studies are needed to establish the use of this laser as an effective and safe treatment modality in nonsurgical periodontics, including repeated interventions of residual pockets after initial periodontal therapy and during the maintenance stage.

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