**ORIGINAL ARTICLE**

# The Effect of ND: YAG Laser on Microorganisms Causing Dental Caries; In Vitro Study

**Ghada Abdul Salam Ibrahim [1, 2*], Intisar Abdel_Jabbar Al Sarraf [1], Bushra Hamza Sadik [1], Safa Thaer Noori [1], Afnan Akram Mohammed [1]**

[1] *Preventive Dentistry, Osol AL-ELM University Collage, Department of Dentistry, Baghdad, Iraq*

[2] *Al-Baladiyat Specialized Dental Centre, Ministry of Health, Baghdad, Iraq*

*Corresponding Author: Ghada Abdul Salam Ibrahim
Email: ghadaalbaghdadi15@gmail.com

Received: 04 January 2025 / Accepted: 11 February 2025

## Abstract

**Purpose:** Streptococcus mutans and Lactobacilli are common microorganisms involved in the caries process, while Candida albicans and Streptococcus mutans are linked to higher rates of tooth decay and severe oropharyngeal conditions. Nd: YAG Lasers are utilized in both medicine and dentistry, enhancing dental enamel's acid resistance and harming pathogenic organisms. This study aims to assess the sensitivity of Candida albicans and Streptococcus mutans to Nd: YAG Laser treatment.

**Materials and Methods:** Microorganisms were exposed to a Q-switched Nd: YAG Laser with a wavelength of 1064 nm, a beam diameter of 4 mm, and energy levels of 100 and 200 mJ for S. mutans, and 300 and 400 mJ for C. albicans. The laser emitted 100 and 200 pulses at a repetition frequency of 3 Hz. All data underwent statistical analysis.

**Results:** Exposing the *Streptococcus mutans* and *Candida albicans* to neodymium dopped-yettrium aluminum garnet, resulted in a reduction in colony forming unit/milliliter (CFU/mL) with increased Laser energy and numbers of pulse with maximum reduction at 200 mJ – 200 pulse and 400 mJ – 200 pulse for *Streptococcus mutans* and *Candida albicans,* respectively. A statistically significant differences were shown in the bacterial number (CFU/mL) between each group when compared with the control group. A statistically significant differences were shown in the fungal number (CFU/mL) between each group in comparison with the control group except between 300mJ-100 pulse and control, there is no significant difference.

**Conclusion:** ND: YAG exhibits antimicrobial effects against Candida albicans and Streptococcus mutans, with a stronger effect on the latter. Additionally, ND: YAG laser therapy promotes wound healing and reduces inflammation, making it an effective tool for managing oral infections. Its deep tissue penetration enables targeted treatment while minimizing harm to surrounding healthy cells. The principle of selective photothermolysis, where the laser energy is preferentially absorbed by pathogens, further enhances its efficacy against these microorganisms.

**Keywords:** ND: YAG Laser; Dental Caries; Microorganisms; *Candida Albicans*.


DOI: https://doi.org/10.18502/fbt.v12i3.19172

## 1. Introduction

Dental caries is a multifactorial disorder involving the collaboration of bacteria on the teeth's surfaces, dental plaque, and diet, particularly carbohydrate parts of meals, which are fermented to organic acids by the plaque microflora over time [1]. Streptococcus mutans and Lactobacilli are common microorganisms in the caries process [2]. Streptococcus mutans adhere to the enamel pellicle and to other plaque microorganism. They are acidogenic and acidouric creating the risk for dental caries [3, 4]. Candida albicans acts as an opportunistic pathogen under certain conditions causes a variety of infections, for instance, thrushes in infants and chronic atrophic candidiasis (denture-induced stomatitis) in adults [5-8]. Candida albicans and Streptococcus mutans are associated with increased tooth caries and severe oropharyngeal illnesses [9, 10]. Studies have confirmed a positive relation between the initiation of dental caries and C. albicans in children and young adults [11-13]. Lasers have many applications in the medical and dental fields [14]. Neodymium-doped Yttrium Aluminum Garnet or Nd: YAG Laser is one of the most famous Laser types. It emits light with an infrared wavelength of 1064 nm. Typically, pulsed Nd: YAG Lasers are triggered in the Q-switching mode [15]. Laser treatment has many advantages in comparison to traditional treatment [16].

Nd: YAG Laser irradiation was able to enhance the dental enamel acid resistance of capable of damaging pathogenic organisms [17-19]. The Laser medicine advancement has resulted in the creation of a number of novel therapeutic approaches capable of eradicating harmful microorganisms. Photoantimicrobial therapy is safe, effective, and easier to administer than standard therapies since it targets viruses, bacteria, fungi, and protozoa [19-21]. The Nd: YAG Laser at 1064 nm was utilized on the idea that its characteristics, such as strong scattering and deep penetration of soft tissue, would be efficient in reducing C. albicans and S. mutans numbers [22, 23]. This experiment sought to determine the sensitivity of S. mutans and C. albicans to the Nd: YAG. Furthermore, this study investigates the antimicrobial efficacy of Nd: YAG lasers against Candida albicans and Streptococcus mutans, two microorganisms associated with dental caries. Using varying laser energies and pulse counts, we observed significant reductions in Colony-Forming Units (CFUs) for both pathogens. Maximum reductions were noted at 200 mJ with 200 pulses for S. mutans and 400 mJ with 200 pulses for C. albicans. These findings suggest that Nd: YAG lasers may serve as effective adjunctive treatments for managing dental caries, warranting further research into their clinical applications and optimal usage parameters.

## 2. Materials and Methods

Nd: YAG Laser radiation effect was tested at different energies and different numbers of pulses on C. albicans and S. mutans and viable counts in this experiment (Figure 1). Pure isolates (for Streptococcus mutans and Candida albicans) were collected from Baghdad Hospital laboratories. The diagnosis depends on biochemical tests, morphological characteristics, and VITEK 2 devices. They were stored in nutritional agar in the refrigerator until needed for the experiment. The activation was made before each experiment by adding 0.1 mL of S. mutans and C. albicans purified isolates in 10 mL of sterilized brain heart infusion broth which incubated aerobically at 37 $^{o}$C for 18 hours. To sterile Ependroff containers, 0.5 mL of each microorganism solution containing 1.5 x $10^{8}$ cells/mL was transferred. These materials were subjected to a Q-switched Nd: YAG Laser with a wavelength of 1064 nm (Model Number: PL755, Wave Length = 1064, 532 nm, China). A beam diameter of 4 mm, and varied energies (100, 200) mJ for S. mutans and (300, 400) mJ for C.albicans. The number of pulses (100, 200) and repetition frequencies (3Hz) (Figure 1). The energy density (fluence) were: 0. 318 J/ $cm^2$ for 100 mJ, 0.636 J/ $cm^2$ for 200 mJ, 0.955 J/ $cm^2$ for 300 mJ, and 1.273 J/ $cm^2$ for 400 mJ. A dilution of $10^{-1}$ was obtained by adding 0.5 mL of irradiation microorganism solution to a test tube containing 4.5 mL of sterilized saline solution. After using the vortex for 15 min to homogenize the solution, from the first tube, 0.5 mL was transmitted to the second tube to obtain a dilution of $10^{-2}$, and this process was repeated until obtained dilution $10^{-6}$.

Then, 0.1 mL of the $10^{-3}$ dilution was transmitted to Mitis Salivarius Bacitracin Agar (MSB Agar) and Sabouraud Dextrose Agar (SDA) Petri dish (at least duplicates). MSB agar is a selective media for the

isolation of streptococci mutans and SDA is a media for the isolation of Candida albicans. As a control, the unirradiated sample was used in this investigation. All MSB Petri dishes were incubated anaerobically for 48-72 hours for MS and SDA were incubated aerobically for 24 hours for C. Albicans. Following an incubation period, the CFU/mL was measured as follows:

Colony Forming Unit (CFU/mL) = Number of colonies x 1/ dilution factor x10 (22).

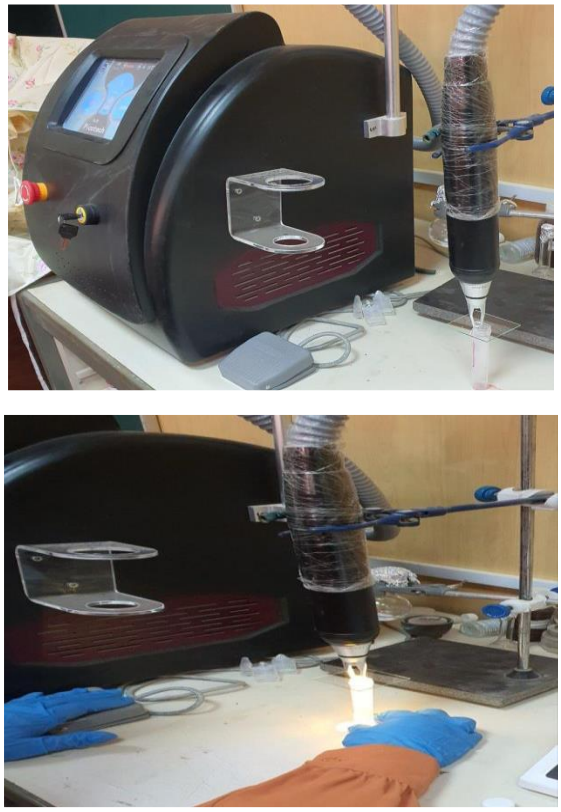

**Figure 1.** Exposure of *streptococci mutans* and *Candida albicans* sample to ND: YAG Laser

## 3. Results

Figure 2 illustrates the sensitivity of Streptococcus mutans to ND: YAG Laser at different energies and number of pulses. The mean and standard deviation of CFU of Streptococcus mutans for the control group (Table 1), (without Laser radiation), and study groups that were exposed to different Laser energy and different numbers of pulses was demonstrated.

Results showed a heavy bacterial growth in the control group, but after Laser exposure, there was a decrease in the CFU/mL mean values for Streptococcus mutans. The maximum reduction was in the group that was irradiated with 200 mJ – 200 pulse. When comparing between groups, there is a significant difference between 100mJ-100 pulse and 200 mJ- 100 pulse, and between 100mJ-200 pulse and 200mJ-200pulse. Between other groups, there is no significant difference (Table 1).

Table 2 presents the multiple comparisons of the bacterial number (CFU/mL) between the control groups with each group using Dunnett 2-sided. There is a significant difference between all groups and control.

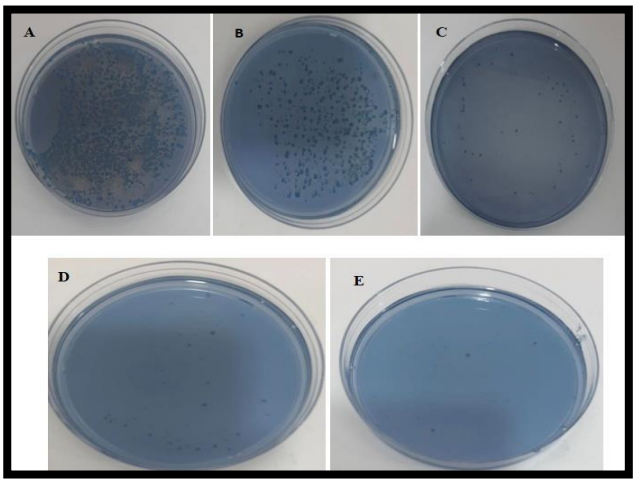


**Figure 2.** Sensitivities of *Streptococcus Mutans* to ND: YAG Laser at different energies and number of pulses (A) control without exposure to Laser (B) exposed to 100mJ-100 pulse. (C) exposed to 100mJ-200 pulse (D) exposed to 200mJ-100 pulse (E) exposed to to200mJ-200 pulse

Figure 3 illustrates the sensitivity of *C. albicans* to ND: YAG Laser at different energies and number of pulses. Table 3, the result of mean and standard deviation of Colony Forming Unit (CFU) of *candida albicans* for the control group (without Laser radiation), and study groups that were exposed to different Laser energies and number of pulses was demonstrated. The result showed a heavy fungal growth in the control group, but after Laser exposure, there was a decrease in the mean values of CFU/mL for *C. albicans*. The maximum reduction was in the group that was irradiated with 400 mJ-200 pulse. There is no significant difference between all study groups except between 300mJ -100 pulse and 400mJ-100 pulse, there is a significant difference. Table 4 demonstrates the multiple Comparisons of the Fungal number (CFU/mL) between the control groups with each group using Dunnett 2-sided. There is a significant difference between all groups and the

**Table 1.** The effect of ND: YAG Laser on *Streptococci mutans* numbers (CFU/mL) among different pulses and energies

| Energy | | Pulses | | | |
|---|---|---|---|---|---|
| | | 100 pulses | 200 pulses | F | P-value |
| 100mJ | Minimum | 36.000 | 18.000 | 1.287 | 0.264 |
| | Maximum | 230.000 | 172.000 | | |
| | Mean | 113.800 | 84.200 | | |
| | ±SD | 74.293 | 61.644 | | |
| | ±SE | 23.494 | 19.493 | | |
| 200mJ | Minimum | 7.000 | 3.000 | 1.738 | 0.196 |
| | Maximum | 170.000 | 19.000 | | |
| | Mean | 46.100 | 11.700 | | |
| | ±SD | 65.249 | 6.165 | | |
| | ±SE | 20.634 | 1.950 | | |
| F | | 6.733 | 7.721 | | |
| P-value | | 0.014* | 0.009* | | |

* = Significant; Significant ($p<0.05$), No significant ($p>0.05$)

**Table 2.** Multiple Comparisons of *Streptococci Mutans* counts after Laser irradiation at different pulses and energy with control by Dunnett 2-sided

| F | P value | Mean±SD of control | (I) Interaction | (J) Interaction | Mean Difference | P value |
|---|---|---|---|---|---|---|
| 16.858 | 0.000* | 209.6±58.110 | 100mJ-100 pulse | Control | -95.800 | 0.002* |
| | | | 100mJ-200 pulse | Control | -125.400 | 0.000* |
| | | | 200mJ-100 pulse | Control | -163.500 | 0.000* |
| | | | 200mJ-200 pulse | Control | -197.900 | 0.000* |

control except between 300mJ-100 pulse and control there is no significant difference.

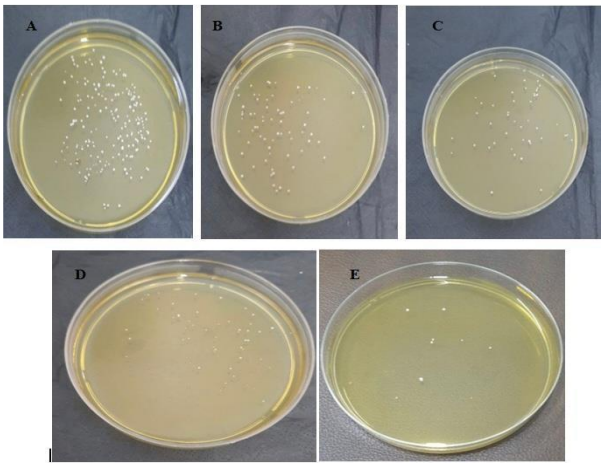


**Figure 3.** Sensitivities of *Candida albicans*to ND: YAG Laser at different energies and number of pulses (A) control without exposure to Laser (B) exposed to 300mJ-100 pulse (C) exposed to 300mJ-200pulse (D) exposed to 400mJ-100 pulse (E) exposed to 400mJ-200 pulse

## 4. Discussion

Our study's findings support previous research on the effectiveness of Nd: YAG lasers in reducing viable microbial counts in dental applications. For instance, Grzech-Leśniak *et al.* (2022) reported significant reductions in C. albicans after laser treatment, particularly in candidiasis cases, reinforcing our conclusion that lasers can aid in managing opportunistic oral infections. In contrast, Kasić *et al.* (2021) found that while Nd: YAG lasers effectively reduced S. mutans counts, the impact on C. albicans was less significant. They suggested that differences in microbial cell absorption characteristics at various laser wavelengths might explain these discrepancies, underscoring the need to optimize laser parameters, such as energy density and exposure time, to enhance antimicrobial efficacy against different pathogens.

Our current study corroborates findings that Nd: YAG laser irradiation significantly reduces the viable count of Streptococcus mutans (1064 nm) [21, 24]. Additionally,

**Table 3.** The effect of ND: YAG Laser on *C. albican numbers* (CFU/mL) among different pulses and energy

| Energy | | Pulses | | | |
|---|---|---|---|---|---|
| | | 100 pulses | 200 pulses | F | P-value |
| 300mJ | Minimum | 70.000 | 37.000 | 2.544 | 0.119 |
| | Maximum | 160.000 | 140.000 | | |
| | Mean | 92.600 | 69.100 | | |
| | ±SD | 35.712 | 38.909 | | |
| | ±SE | 11.293 | 12.304 | | |
| 400mJ | Minimum | 34.000 | 31.000 | 0.407 | 0.528 |
| | Maximum | 110.000 | 100.000 | | |
| | Mean | 61.400 | 52.000 | | |
| | ±SD | 29.432 | 26.192 | | |
| | ±SE | 9.307 | 8.283 | | |
| F | | 4.484 | 1.347 | | |
| P-value | | 0.041* Sig. | 0.253 NS | | |

**Table 4.** Multiple Comparisons of C*andida albican* counts after Laser irridation at different pulses and energy with control by Dunnett 2-sided

| F | P-value | Mean±SD of control | (I) Interaction | (J) Interaction | Mean Difference | P-value |
|---|---|---|---|---|---|---|
| 5.921 | 0.001* Sig. | 127.6±58.399 | 300mJ-100 pulse | Control | -35.000 | 0.162 |
| | | | 300mJ-200 pulses | Control | -58.500 | 0.006* |
| | | | 400mJ-100 pulse | Control | -66.200 | 0.002* |
| | | | 400mJ-200 pulse | Control | -75.600 | 0.000* |

the reduction in Candida counts after Nd: YAG laser treatment aligns with Grzech-Leśniak *et al.*, indicating that laser antimicrobial treatment can notably decrease C. albicans, especially in managing candidiasis and caries [20]. However, unlike Kasić *et al.*, we found no statistically significant decrease in C. albicans CFUs after Nd: YAG laser irradiation [9, 25], potentially due to variations in laser parameters and sample differences. The Near-Infrared (NIR) laser may inhibit and kill S. mutans through a wavelength-dependent photothermal interaction mechanism, where longer wavelengths and lower energy cause molecular oscillation. This vibrational energy is converted into kinetic energy through molecular collisions, resulting in increased kinetic energy and thermal stress, known as photothermolysis [25].

Moreover, the thermal effects of Nd: YAG lasers on microbial cells are well-documented; studies have shown that photothermal interaction leads to cellular damage through increased kinetic energy and subsequent thermal stress, resulting in the death of the targeted microorganisms [26]. Our study corroborates these findings, as we observed a clear relationship between increased laser energy, pulse counts, and reductions in CFUs.

Thermal interaction refers to biological processes that occur inside cells as a result of temperature changes. These processes include reduced enzyme activity, denaturation of proteins, cell immobility, increased vaporization, permeability, and thermal decomposition leading to high damage and burst cells which can happen at high temperatures [27, 28]. These biological effects are brought on by the NIR wavelength matching biological acceptor molecules, such as water molecules, or other macromolecules, such as pigments and proteins [29]. Nd: YAG Laser light at 1064 nm photoexcited the endogenous microbial porphyrin molecules found in C. albicans and S. mutans and by, which causes oxidative damage through Reactive Oxygen Species (ROS) [24].

Another explanation is that Nd: YAG Laser 1064 nm has many characteristic qualities, deep soft tissue penetration, and high scattering effect, and this may be more effective in inhibition of Candida albicans and Streptococcus. mutans [22, 24, 30]. The susceptibilities of the two tested pathogens to Nd:YAG irradiation differ in this study. Nd: YAG is more effective on S. mutans than on the candida. This may be wavelength of Nd: YAG does not get absorbed well by candida and it may need higher irradiance values to increase the antimicrobial effect against candida [31].

One significant advantage of Nd: YAG laser treatment is its ability to penetrate soft tissues and affect bacteria located deep within dental biofilms. This characteristic could make it a valuable tool for dental professionals, especially since traditional mechanical methods may not reach certain areas effectively [32].

Furthermore, while the current study focused on the in vitro effects, there is a pressing need to explore the clinical applications of Nd: YAG lasers. Studies have suggested that laser therapy may enhance the remineralization of dental enamel, potentially offering a dual benefit of reducing microbial loads while simultaneously promoting tooth health [33].

In conclusion, while our findings add to the growing body of evidence supporting the use of Nd: YAG lasers in dentistry, further clinical trials are necessary. Understanding the precise mechanisms by which lasers interact with various microorganisms and assessing their long-term effects will pave the way for integrating this technology into routine dental care effectively.

## 5. Conclusion

In summary, this study demonstrates that Nd: YAG laser treatment exhibits significant antimicrobial activity against Candida albicans and Streptococcus mutans, with a greater efficacy observed against S. mutans. The findings indicate that increasing laser energy and pulse count enhances the reduction of colony-forming units (CFUs) for both microorganisms. Specifically, maximum reductions were achieved at 200 mJ with 200 pulses for S. mutans and 400 mJ with 200 pulses for C. albicans.

The potential implications of these results are substantial, as they suggest that Nd: YAG lasers could serve as an effective adjunctive treatment to traditional methods for managing dental caries. Future research should explore the in vivo efficacy, optimal treatment parameters, and long-term outcomes of Nd: YAG lasers in clinical settings. Additionally, further investigations are needed to understand the mechanisms by which laser energy affects different microbial populations and to assess the safety and effectiveness of using lasers in diverse dental applications.

## Acknowledgment

Ethical approval was administrated at the College of Dentistry, the Department of Pediatric and Preventive, University of Baghdad, Ethical Committee (Ref. 560 on April 2022). in compliance with the guiding principles of the Helsinki Declaration [34].

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