Journal of Lasers in Medical Sciences

J Lasers Med Sci. 2025;16:e29

Original Article

http://journals.sbmu.ac.ir/jlms

doi 10.34172/jlms.2025.29

Comparing the Effect of Photodynamic Therapy With 660 and 450 nm Lasers and Cold Atmospheric Plasma on *Candida albicans*: An Invitro Study

Arash Azizi[1], Seyedeh Elnaz Mousavi Zadeh[2], Seyed Khalil Shokouhi Mostafavi[3], Mohammad Vahedi[1*]

[1]Department of Oral and MaxilloFacial Medicine, TeMS.C., Islamic Azad University, Tehran, Iran
[2]Specialist and Board Certified in oral and MaxilloFacial Medicine, Private Practice, Tehran, Iran
[3]Department of Microbiology, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

***Correspondence to**
Mohammad Vahedi,
Email: Vahedi_md@yahoo.com

Received: August 18, 2024
Accepted: July 12, 2025
ePublished: September 2, 2025

Abstract

Introduction: The increasing occurrence of antifungal resistance agents poses a significant challenge in the field of dentistry, necessitating new therapeutic alternatives for treating fungal infections. This study compares the effect of photodynamic therapy (PDT) using two wavelengths of diode lasers and cold atmospheric plasma (CAP) on *Candida albicans* cultures.

Methods: A standard 0.5 McFarland suspension of *C. albicans* was prepared and transferred to a 96-well microplate. These samples were divided into 10 groups as follows: 1- Positive control, 2- 450 nm laser, 3- PDT with 450 nm laser + curcumin, 4- 660 nm laser, 5- PDT with 660 nm laser + methylene blue, 6 and 7- Cold plasma for 180 and 210 seconds, 8- Nystatin, 9- Curcumin, 10- Methylene blue. Then the colony counts of *C. albicans* (CFU/mL) were recorded. Data analysis was performed using SPSS software, One way ANOVA, and Tamhane's test ($P<0.05$).

Results: The mean reduction in the number of *C. albicans* colonies in the nystatin group was significantly higher than all other groups ($P<0.05$). The 660 nm laser group showed significant reduction in the colony count compared to the positive control, 210-second cold plasma, and 450 nm PDT groups ($P<0.05$). The 180-second cold plasma group had significantly higher reduction in the colony count compared to the positive control and 450 nm PDT groups ($P<0.05$). The methylene blue, 450 nm laser, and 210-second cold plasma groups also showed significantly higher reductions compared to the positive control ($P<0.05$).

Conclusion: Cold plasma with the feeding gas of helium for 180 seconds exposure and the low-power 660 nm laser showed inhibitory effects on *C. albicans* colonies and were more effective than PDT. Nystatin application significantly reduced the number of *C. albicans* colonies and was more effective than cold plasma, laser irradiation, and PDT.

Keywords: Cold plasma; *Candida albicans*; Photodynamic therapy; Laser.

Introduction

In recent decades, the prevalence of fungal infections has increased in many countries. Due to the emergence of resistance to antifungal agents, developing an effective strategic program is crucial for treating fungal infections in clinical mycology.[1,2] Oral candidiasis is an opportunistic fungal infection that typically affects the oral mucosa.[3,4] The primary causative agent is *Candida albicans* (responsible for 60-70% of cases isolated from the mouth), with other *Candida* species such as *Candida glabrata, Candida parapsilosis, Candida krusei,* and *Candida tropicalis* also being involved.[5] *Candida albicans* is a highly diverse organism well-adapted to its human host and is the most common fungal species in human microbiota, colonizing about 50% of healthy individuals. It can become pathogenic under favorable conditions, usually involving a disruption in the anatomical barrier function or a microbial flora imbalance. The occurrence of infection depends on the complex interaction between fungal virulence factors, such as surface adhesion, proteolytic enzymes, morphological changes, and drug resistance, along with the host's immune system.[3,6] Traditionally, various antifungal drugs, including miconazole, fluconazole, clotrimazole, ketoconazole, and nystatin, are used topically or systemically to treat candidiasis. Despite their effectiveness, these drugs have numerous side effects, such as anemia, hypokalemia, fever, and nausea. Additionally, the treatment duration for fungal infections can be prolonged.[1] The increasing resistance to antifungal drugs like amphotericin B and fluconazole poses further challenges.[7] Alternative therapeutic methods being used include tea tree oil,[8] lemon oil,[9] oregano oil,[10] colloidal metal nanoparticle solutions (silver, gold),[11] ozone therapy,[12,13] photobiomodulation,[14,15] antimicrobial

Please cite this article as follows: Azizi A, Mousavi Zadeh SE, Shokouhi Mostafavi SK, Vahedi M. Comparing the effect of photodynamic therapy with 660 and 450 nm lasers and cold atmospheric plasma on *Candida albicans*: an in vitro study. *J Lasers Med Sci.* 2025;16:e29. doi:10.34172/jlms.2025.29.

photodynamic therapy (aPDT),[16,17] and cold atmospheric plasma (CAP).[1] Photodynamic therapy (PDT) is a new therapeutic strategy based on the interaction of a non-toxic photosensitizer (PS) and a harmless light source, which, in the presence of oxygen, produces reactive oxygen species (ROS), leading to biological events culminating in apoptosis and microorganism death.[18] PSs have minimal cellular toxicity but, when activated by appropriate light wavelengths, generate ROS.[19] Therefore, PDT has been successfully used to treat *C. albicans*.[19,20] On the other hand, CAP has emerged as a promising and innovative technology for biomedical applications in recent years. Although the precise microbial inactivation mechanism of CAP is not fully understood, it is attributed to the synergistic action of reactive oxygen and nitrogen species (RONS) which is generated by the ionization of a noble gas and therefore has an antimicrobial effect.[6] CAP's inhibitory effect on planktonic *Candida* cultures and biofilms has been reported, and it enhances the effectiveness of conventional antifungal drugs.[21-24] However, its application has not been evaluated alongside PDT. Therefore, the present study aims to compare the effect of PDT with two wavelengths (660 nm and 450 nm) of diode lasers and CAP on *C. albicans* under laboratory conditions at Tehran Islamic Azad University of Medical Sciences.

Methods

Fungal Strain and Culture Conditions

In this experimental study, *C. albicans* strain ATCC 14053 was obtained from the Iranian Biological Resource Center and cultured as a suspension in Sabouraud dextrose agar (SDA). After ensuring culture purity, a standard 0.5 McFarland suspension (CFU/mL 1.5×10^8) was prepared and transferred to a 96-well microplate (0.1 mL per well).[19,25]

Curcumin aqueous extract with a concentration of 10.2% (Adonis Gol Daru Company, Alborz, Iran) and methylene blue (Merck KGaA, Darmstadt, Germany) with a concentration of 0.02% were diluted using physiological serum. Then, 0.1 mL of *C. albicans* suspension was placed inside a 96-well microplate using a sampler. Based on the type of sample group, the same amount of sterile physiological serum or PS was added. All these steps were performed under a laminar hood to provide a dark and sterile environment.[25]

Cold Atmospheric Plasma Device

Plasma application was performed using the PlasmArt device (NariaTech, Tehran, Iran). In this device, gas flow is ionized in a dielectric chamber to produce cold plasma. Helium was used as the feeding gas at an inlet pressure of 4.5 bar, with an outlet pressure of atmospheric pressure and a gas flow rate of 3.8 L/min and intensity of 4 (0.780 kV). The device had an 8-watt power output and a 100 kHz handpiece frequency. The plasma jet length was 21 mm with a diameter of 2.5 mm. Plasma irradiation was conducted by positioning the plasma jet nozzle on the surface of the well samples.

Sample Volume and Groups

Using one way ANOVA analysis and PASS 11 software, with $\alpha = 0.05$, $\beta = 0.1$, a standard deviation of 2.08 for the log of *C. albicans* colonies, and an effect size of 0.64, the minimum sample size for each of the 10 study groups was 8 samples. *Candida albicans* samples were divided into following 10 groups:

1. Positive control: No treatment was performed in this group. The wells had 0.5 McFarland *C. albicans* without using any type of therapeutic intervention.

2. 450 nm laser irradiation: The samples were irradiated with a 450 nm diode laser (Sirona, Germany) at 100 mW power and 12 J/cm^2 energy density for 60 seconds without a PS (PS), using sterile saline instead.[25] The diameter of the low power head in all laser groups was 0.8 cm. Also, the laser was irradiated perpendicular to the surface of the suspension in the opening of the wells. In each sample well, two empty wells were considered and these empty wells were also covered with foil paper in order to minimize the effect of radiation on the adjacent sample wells.

3. 450 nm PDT: Curcumin (10.2 mg/cc) was added, and the samples were irradiated with a 450 nm diode laser at 100 mW power and 12 J/cm2 energy density for 60 seconds.[25]

4. 660 nm laser irradiation: The samples were irradiated with a 660 nm diode laser (Sirona, Germany) at 100 mW power and 20 J/cm^2 energy density for 100 seconds without a PS, using sterile saline instead.[25]

5. 660 nm PDT: Methylene blue (0.02%) was added, and the samples were irradiated with a 660 nm diode laser at 100 mW power and 20 J/cm^2 energy density for 100 seconds.[25]

6 and 7. Cold atmospheric plasma: 10 µL of cell suspension was poured into the selected wells of 96-well microtiter plates. Cold plasma was irradiated to each group of samples for 180 and 210 seconds with the mentioned conditions.[1,26]

8. Nystatin: 0.1 mL of nystatin (100,000 units) (Jaber-ebn-Hayan Pharmaceuticals, Tehran, Iran) was added to the samples.[25]

9. Curcumin: Curcumin (10.2 mg/cc) was added to the samples.

10. Methylene blue: Methylene blue (0.02%) was added to the samples.

Evaluation

In the next step, suspensions in each well were cultured on SDA, and the results were reported after 24 hours of incubation at 37 °C. Colony counts (CFU/mL) were

performed post-incubation. In the nystatin group, the pour plate technique was used to count *C. albicans* colonies in dilute suspension. For this purpose, 0.1 mL of the suspension was poured into an empty sterile plate by a sampler. 25 mL of cooled sterile SDA was added, and after closing the lid, the plate was slowly rotated to mix the sample and culture medium. Then the plate was transferred to the incubation device. Colonies (CFU/mL) were counted in all samples after 24 hours of incubation in the SDA culture medium at 37 °C.[19,25]

Data Analysis

Data were analyzed using SPSS version 26. The Kolmogorov-Smirnov test was used for normality assessment. One way ANOVA and Tamhane's test were used for data analysis. Values less than 0.05 were considered significant.

Results

The number of *C. albicans* colonies (CFU/mL) in the ten groups was evaluated. Table 1 shows the mean colony counts in the groups after intervention, so the difference between before and after the intervention of each group is considered by *P* value. The lowest colony counts were observed in the following order: Nystatin, 660 nm laser, 450 nm laser, methylene blue, 660 nm PDT, 180-second cold plasma, 450 nm PDT, 210-second cold plasma, curcumin, and positive control. According to the one-way ANOVA test, there was a significant difference in the mean colony counts between the groups ($P<0.05$). Also, Figure 1 shows the mean and standard deviation of the number of *C. albicans* colonies (CFU/mL) in the studied groups before and after the intervention. According to the Tamhane test, pairwise comparisons showed that the mean colony counts in the nystatin group were significantly lower than those in all other groups ($P<0.05$). The mean colony count in the 660 nm laser group was significantly lower than in the positive control, 210-second cold plasma, and 450 nm PDT groups ($P<0.05$). However, no significant difference was found between this group and the 450 nm laser, 660 nm PDT, 180-second cold plasma, curcumin, and methylene blue groups ($P>0.05$) (Table 2). As we showed in table 3, the mean percentage reduction in colony counts for the 180-second cold plasma group was significantly higher than the positive control and 450 nm PDT groups ($P<0.05$). The mean percentage reduction in colony counts for the methylene blue, 450 nm laser, and 210-second cold plasma groups was significantly higher than the positive control ($P<0.05$). However, the mean percentage of colony reduction in the positive control group was not significantly different from curcumin, PDT with 450 nm, and PDT with a 660 nm wavelength ($P>0.05$). PDT with a wavelength of 450 nm and PDT with a wavelength of 660 nm had the same effect as the positive control ($P>0.05$). Other group comparisons were not significant ($P>0.05$). The mean percent reduction in the number of *C. albicans* colonies (CFU/mL) in the studied groups is presented in Table 3. The highest mean percentages of *C. albicans* colony reduction (CFU/mL) across the groups were respectively as follows: nystatin, laser radiation with a wavelength of 660 nm, cold plasma radiation for 180 seconds, methylene blue, laser radiation with a wavelength of 450 nm, cold plasma radiation for 210 seconds, curcumin, PDT with a wavelength of 450 nm, PDT with a wavelength of 660 nm, and positive control. Based on the One-way ANOVA test, the mean percentage of *C. albicans* colony reduction (CFU/mL) in the studied groups had a significant difference ($P<0.05$).

Table 1. Mean Colony Counts of *Candida albicans* (CFU/mL) Post-intervention

Groups	Number	CFU/mL Post-Intervention (Mean ± SD)	*P* value
Nystatin	8	65.75 ± 20.852	<0.001
660 nm Laser irradiation	8	162.38 ± 9.288	<0.001
450 nm Laser irradiation	8	168.88 ± 7.568	<0.001
Methylene blue	8	175.50 ± 39.042	<0.001
660 nm PDT	8	192.38 ± 43.782	<0.001
180-Second cold plasma	8	192.63 ± 42.285	<0.001
450 nm PDT	8	211.38 ± 22.129	<0.001
210-Second cold plasma	8	225.13 ± 13.799	<0.001
Curcumin	8	230.50 ± 48.000	<0.001
Positive control	8	233.50 ± 11.637	<0.001

Discussion

Oral candidiasis is an opportunistic infection caused by the overgrowth of *C. albicans* and other Candida species, common in elderly individuals, denture wearers, and immunocompromised patients, and it can be a sign of systemic diseases.[27] The rise in antifungal resistance agents necessitates new therapeutic approaches against Candida infections to achieve better treatment outcomes without systemic side effects.[26] This study compared the effects of PDT with two wavelengths (660 nm and 450

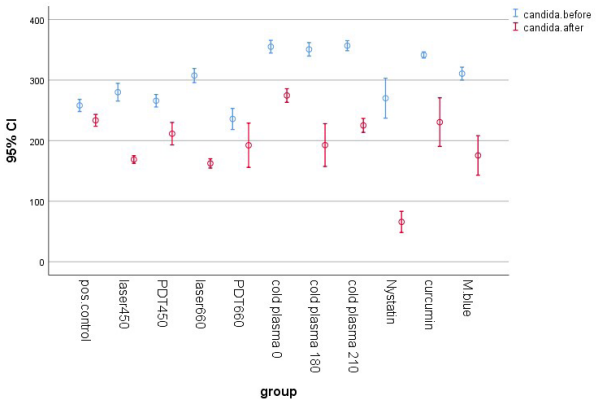

Figure 1. The Mean and Standard Deviation of the Number of *Candida albicans* Colonies (CFU/mL) in the Investigated Groups

Table 2. Pairwise Comparison of the Mean Colony Counts of *Candida albicans* (CFU/mL) in the Studied Groups After the Intervention

Groups	Groups	*P* Value
Nystatin	Positive control	0.000*
	Laser irradiation with a wavelength of 450 nm	0.000*
	Photodynamic therapy with a wavelength of 450 nm	0.000*
	Laser irradiation with a wavelength of 660 nm	0.000*
	Photodynamic therapy with a wavelength of 660 nm	0.001*
	Cold plasma irradiation for 180 seconds	0.001*
	Cold plasma irradiation for 210 seconds	0.000*
	Curcumin	0.000*
	Methylene blue	0.001*
Laser irradiation with a wavelength of 660 nm	Positive control	0.000*
	Laser irradiation with a wavelength of 450 nm	1.000
	Photodynamic therapy with a wavelength of 450 nm	0.012*
	Photodynamic therapy with a wavelength of 660 nm	0.996
	Cold plasma irradiation for 180 seconds	0.992
	Cold plasma irradiation for 210 seconds	0.000*
	Curcumin	0.234
	Methylene blue	1.000
Laser irradiation with a wavelength of 450 nm	Positive control	0.000*
	Photodynamic therapy with a wavelength of 450 nm	0.038*
	Photodynamic therapy with a wavelength of 660 nm	1.000
	Cold plasma irradiation for 180 seconds	1.000
	Cold plasma irradiation for 210 seconds	0.000*
	Curcumin	0.364
	Methylene blue	1.000
Methylene Blue	Positive control	0.179
	Photodynamic therapy with a wavelength of 450 nm	0.920
	Photodynamic therapy with a wavelength of 660 nm	1.000
	Cold plasma irradiation for 180 seconds	1.000
	Cold plasma irradiation for 210 seconds	0.370
	Curcumin	0.757
Photodynamic therapy irradiation with a wavelength of 660 nm	Positive control	0.845
	Photodynamic therapy with a wavelength of 450 nm	1.000
	Cold plasma irradiation for 180 seconds	1.000
	Cold plasma irradiation for 210 seconds	0.988
	Curcumin	0.999
Cold plasma for a duration of 180 seconds	Positive control	0.810
	Photodynamic therapy with a wavelength of 450 nm	1.000
	Cold plasma irradiation for 210 seconds	0.982
	Curcumin	0.999
Photodynamic therapy irradiation with a wavelength of 450 nm	Positive control	0.814
	Cold plasma irradiation for 210 seconds	1.000
	Curcumin	1.000
Cold plasma for a duration of 210 seconds	Positive control	1.000
	Curcumin	1.000
Curcumin	Positive control	1.000

* Significant difference at the 0.05 level based on the Tamhane test.

Table 3. Mean Percentage Reduction in the Colony Counts of *Candida albicans* (CFU/mL)

Groups	Number	CFU/mL (Mean ± SD) (%)	*P* value
Nystatin	8	75.49 ± 7.07	<0.001
660 nm Laser irradiation	8	47.05 ± 4.45	<0.001
180-Second cold plasma	8	45.18 ± 10.97	<0.001
Methylene blue	8	43.08 ± 14.18	<0.001
450 nm Laser irradiation	8	39.37 ± 6.15	<0.001
210-Second cold plasma	8	36.90 ± 3.24	<0.001
Curcumin	8	32.38 ± 14.51	<0.001
450 nm PDT	8	20.09 ± 11.55	<0.001
660 nm PDT	8	17.77 ± 20.13	<0.001
Positive control	8	9.39 ± 5.68	<0.001

nm) of diode lasers and CAP on *C. albicans*. Results showed a significant reduction in *C. albicans* colonies with different exposure times to cold helium plasma compared to the control. But the effect of 180-second cold plasma was similar to 210-second exposure. Various studies have reported the effectiveness of cold plasma on *C. albicans*. Borges et al found that 300-second helium plasma exposure could eliminate *C. albicans* biofilm.[28] Also, in another study, by making changes in CAP (plasma jet with amplitude modulated voltage signal (AM-CAPPJ)), these researchers investigated its effect in vivo on the mouse model and found that the application of CAP for 5 minutes caused the survival of *C. albicans* decrease compared to the non-irradiated group.[6] Rahimi-Verki et al also observed a reduction in *C. albicans* colonies with 180-second CAP exposure.[1] Ebrahimi-Shaghaghi et al reported that the application of DBD with helium gas after 180 and 210 seconds inhibited the growth of *C. albicans* (planktonic cells) compared to the control group.[26] Leite et al found that 5-minute DBD helium plasma exposure inhibited *C. albicans* biofilm.[2] Wanachantararak et al observed a significant colony reduction in *C. albicans* with 10-minute DBD argon plasma exposure, with 15-minute exposure showing results similar to the control.[29] Despite differences in devices, gases, and exposure times, these studies align with the current study's findings. However, it should be kept in mind that toxic effects usually appear after longer exposure periods.[6,30,28] A previous study showed that after 5 minutes of cold plasma application, the viability of microbial cells is less than 45%.[30] In addition, another study reported that cell viability was less than 5% after 5 minutes of exposure to a helium plasma jet operated with a sine wave voltage signal.[28] The mechanism of plasma effect against fungi was investigated by Misra et al. These researchers found that the mechanisms of CAP effect for the inactivation of fungi include the following: inhibiting the function of cell membrane due to the structural changes and increasing its permeability resulting severe morphological changes and finally apoptosis, in addition, production of ROS by cold plasma causes the oxidation of intracellular organelles and cell death.[31] Ebrahimi-Shaghaghi et al also observed changes by the SEM microscope, the upward trend of damage to cell membrane structure, change in cell wall thickness, and mitochondrial structure swelling, after CAP radiation. Also, an increase in the number of vacuoles and acidification due to the external leakage of the cell cytoplasm was observed in the treatments of 180 and 210 seconds.[26] The effect of cold plasma on the pathogenic factors of *C. albicans* such as filament formation,[32] adhesion and reduction of ergosterol production, and reduction of biofilm production has also been reported.[1,32] The present study showed that PDT with a 660 nm laser with methylene blue (0.02%), 660 nm laser alone, and 450 nm laser alone had similar effects on reducing *C. albicans* colonies. Also, PDT with a wavelength of 450 nm with curcumin had the same effect as the positive control. Unlike the present study, Daliri et al found that PDT with a 460 nm laser with curcumin was more effective than PDT with a 660 nm laser with methylene blue in reducing *C. albicans* colonies.[25] Ma et al reported that PDT with a 455 nm laser inhibited *C. albicans* biofilm at various energy densities (13.2, 10.56, 7.92, 5.28 and 2.64 J/cm^2).[33] Also, in the study of Marques Meccatti et al, a further reduction in CFU/mL was observed using a PDT protocol with curcumin (110 mW/cm^2; 25 J/cm^2 for 228 seconds) with a long-term dose, especially in *C. albicans*.[34] Considering that the laser acts with a dose-dependent behavior in causing changes in the fungus *C. albicans*, the difference in dosimetry between these studies and the present study can be the reason for obtaining different results in this field.[35] The concentration of methylene blue can be one of the influential factors regarding the ineffectiveness of PDT. In the present study, the concentration of 0.02% methylene blue was used,[19] while Heidari et al evaluated the effect of three concentrations of 0.1, 0.01 and 0.001 g/L of methylene blue and found that only the concentration of 0.001 was effective in reducing the *C. albicans* colony.[35] On the other hand, fungi are generally less sensitive to aPDT than bacteria, which may be due to their larger cell size and lower ROS target sites.[36] According to studies, curcumin absorbs light in the entire blue visible spectrum, in the range of 300 to 500 nm, and its maximum absorption, depending on the solvents used, is on average in the range of 418 nm. Also, curcumin, as a lipophilic molecule, directly interacts with the membrane and membrane proteins.[34] The results of the present study showed that 660 nm laser irradiation alone effectively reduced *C. albicans* colonies. In the study of Daliri et al, the use of 660 nm diode lasers (with power of 10 mW, 100 mW) and 460 nm (with power of 25 mW) at times of 30 and 60 seconds caused a significant reduction in the standard *C. albicans* colony compared to the control group.[25] In the study of

Wiench et al, they found that 635 nm diode laser radiation (400mW, 30s, 24 J/cm^2) caused a significant reduction in *C. albicans* biofilm.[37] Seyedmousavi et al exposed *C. albicans* cultures to energy levels of 3, 5, 10, and 20 J at a wavelength of 685 (50 mW). The researchers concluded that the laser at an energy level higher than 10 J at 685 nm wavelengths can significantly reduce the growth of *C. albicans* colonies.[38] In the study of Souza et al, the effect of low-power laser radiation with a wavelength of 660 nm (Ga-AL-Ar laser) was investigated and a decrease in candida was observed.[39] Queiroga et al compared the 660 nm laser with the energy densities of 60, 120 and 180 J/cm^2 and the power of 40 mW and the times of 1, 2 and 3 minutes against different species of Candida in comparison with the control group, and the results showed that the 660 nm laser caused a significant reduction in the colony in all three energy densities.[40] This study was similar to the present study in terms of the type of wavelength, and the findings of these studies were in line with the present study. The results of this study showed that the use of nystatin significantly reduced *C. albicans* colonies by 75%, more effectively than cold plasma, laser irradiation, and PDT. This finding was in line with the study of Azizi et al who found that PDT with a 660 nm laser with methylene blue and a 660 nm laser alone were not more effective than nystatin.[19] Unlike the present study, Leite et al reported that 5-minute DBD plasma was more effective than nystatin and amphotericin B in *C. albicans* biofilm.[2] Borges et al also reported that plasma and nystatin reached similar levels after 48 hours of exposure in terms of reducing cell invasion in a mouse model of candidiasis.[6] Azizi et al found that PDT with a 460 nm laser with curcumin was more effective than nystatin in eliminating *Candida*.[19] Differences in nystatin concentration and irradiation parameters could explain the varying results. Nystatin's positive effect on reducing *C. albicans* biofilm has been reported in other studies.[2,41] Nystatin, a topical antifungal, is the gold standard for candidiasis treatment.[42] Nystatin is obtained from *Streptomyces noursei* and can bind to the ergosterol of the plasma membrane of the fungus and create pores that make it more permeable and cause the loss of intracellular potassium with a fungicidal effect.[27] Ergosterol is responsible for maintaining the integrity of the cell wall. In addition, nystatin causes secondary cell damage by autoxidation. The anti-candidacy spectrum of nystatin is very wide.[26-27,43,44] Therefore, the results of the present study indicate that despite the emergence of new and various treatment methods for *C. albicans*, nystatin is still used as the first line and the most effective method.

Conclusion

CAP exposure with helium gas for 180 seconds and low-power 660 nm laser irradiation showed more effective inhibitory effects on *C. albicans* colonies in comparison with PDT. Nystatin application significantly reduced colony counts and was more effective than cold plasma, low power laser irradiation, and PDT. However, it is suggested to conduct other studies with more details in this field.

Acknowledgements

This study was derived from a thesis submitted in partial fulfillment of the requirements for a master's degree in Oral and Maxillofacial Medicine from the Faculty of Dentistry, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran, and there was no conflict of interest.

Authors' Contribution

Conceptualization: Mohammad Vahedi, Arash Azizi.
Data curation: Mohammad Vahedi, Arash Azizi.
Formal analysis: Mohammad Vahedi, Arash Azizi, Seyed Khalil Shokouhi Mostafavi.
Investigation: Mohammad Vahedi, Arash Azizi, Seyedeh Elnaz Mousavi Zadeh.
Methodology: All authors
Project administration: Mohammad Vahedi, Arash Azizi, Seyed Khalil Shokouhi Mostafavi.
Resources: Seyedeh Elnaz Mousavi Zadeh.
Software: Seyedeh Elnaz Mousavi Zadeh.
Supervision: Mohammad Vahedi, Arash Azizi.
Validation: Mohammad Vahedi, Arash Azizi.
Visualization: Mohammad Vahedi, Arash Azizi, Seyed Khalil Shokouhi Mostafavi.
Writing–original draft: Mohammad Vahedi.
Writing–review & editing: Mohammad Vahedi.

Competing Interests

None to declare.

Ethical Approval

This In-vitro research was registered with the ethics code of IR.IAU.TMU.REC.1401.226 on 20/02/1401. This research was registered with the ethics code IR.IAU.TMU.REC.1401.226 on 20/02/1401.

Funding

None.

References

1. Rahimi-Verki N, Shapoorzadeh A, Razzaghi-Abyaneh M, Atyabi SM, Shams-Ghahfarokhi M, Jahanshiri Z, et al. Cold atmospheric plasma inhibits the growth of *Candida albicans* by affecting ergosterol biosynthesis and suppresses the fungal virulence factors in vitro. Photodiagnosis Photodyn Ther. 2016;13:66-72. doi: 10.1016/j.pdpdt.2015.12.007.
2. Leite LD, de Oliveira MA, da Cruz Vegian MR, da Graça Sampaio A, Nishime TM, Kostov KG, et al. Effect of cold atmospheric plasma jet associated to polyene antifungals on *Candida albicans* biofilms. Molecules. 2021;26(19):5815. doi: 10.3390/molecules26195815.
3. Vila T, Sultan AS, Montelongo-Jauregui D, Jabra-Rizk MA. Oral candidiasis: a disease of opportunity. J Fungi (Basel). 2020;6(1):15. doi: 10.3390/jof6010015.
4. Contaldo M, Di Stasio D, Romano A, Fiori F, Della Vella F, Rupe C, et al. Oral Candidiasis and Novel Therapeutic Strategies: Antifungals, Phytotherapy, Probiotics, and Photodynamic Therapy. Curr Drug Deliv. 2023;20(5):441-56. doi: 10.2174/1567201819666220418104042.
5. Wiench R, Skaba D, Matys J, Grzech-Leśniak K. Efficacy of toluidine blue-mediated antimicrobial photodynamic therapy

on *Candida* spp. A systematic review. Antibiotics (Basel). 2021;10(4):349. doi: 10.3390/antibiotics10040349.
6. Borges AC, de Morais Gouvêa Lima G, Nishime TM, Gontijo AV, Kostov KG, Koga-Ito CY. Amplitude-modulated cold atmospheric pressure plasma jet for treatment of oral candidiasis: in vivo study. PLoS One. 2018;13(6):e0199832. doi: 10.1371/journal.pone.0199832.
7. Maisch T, Shimizu T, Isbary G, Heinlin J, Karrer S, Klämpfl TG, et al. Contact-free inactivation of *Candida albicans* biofilms by cold atmospheric air plasma. Appl Environ Microbiol. 2012;78(12):4242-7. doi: 10.1128/aem.07235-11.
8. Ninomiya K, Maruyama N, Inoue S, Ishibashi H, Takizawa T, Oshima H, et al. The essential oil of *Melaleuca alternifolia* (tea tree oil) and its main component, terpinen-4-ol protect mice from experimental oral candidiasis. Biol Pharm Bull. 2012;35(6):861-5. doi: 10.1248/bpb.35.861.
9. Wright SC, Maree JE, Sibanyoni M. Treatment of oral thrush in HIV/AIDS patients with lemon juice and lemon grass (*Cymbopogon citratus*) and gentian violet. Phytomedicine. 2009;16(2-3):118-24. doi: 10.1016/j.phymed.2008.07.015.
10. Pradebon Brondani L, Alves da Silva Neto T, Antonio Freitag R, Guerra Lund R. Evaluation of anti-enzyme properties of *Origanum vulgare* essential oil against oral *Candida albicans*. J Mycol Med. 2018;28(1):94-100. doi: 10.1016/j.mycmed.2017.12.001.
11. Jebali A, Haji Esmaeil Hajjar F, Pourdanesh F, Hekmatimoghaddam S, Kazemi B, Masoudi A, et al. Silver and gold nanostructures: antifungal property of different shapes of these nanostructures on *Candida* species. Med Mycol. 2014;52(1):65-72. doi: 10.3109/13693786.2013.822996.
12. Arita M, Nagayoshi M, Fukuizumi T, Okinaga T, Masumi S, Morikawa M, et al. Microbicidal efficacy of ozonated water against *Candida albicans* adhering to acrylic denture plates. Oral Microbiol Immunol. 2005;20(4):206-10. doi: 10.1111/j.1399-302X.2005.00213.x.
13. Zargaran M, Fatahinia M, Zarei Mahmoudabadi A. The efficacy of gaseous ozone against different forms of *Candida albicans*. Curr Med Mycol. 2017;3(2):26-32. doi: 10.18869/acadpub.cmm.3.2.26.
14. Dompe C, Moncrieff L, Matys J, Grzech-Leśniak K, Kocherova I, Bryja A, et al. Photobiomodulation-underlying mechanism and clinical applications. J Clin Med. 2020;9(6):1724. doi: 10.3390/jcm9061724.
15. Grzech-Leśniak K, Nowicka J, Pajączkowska M, Matys J, Szymonowicz M, Kuropka P, et al. Effects of Nd:YAG laser irradiation on the growth of *Candida albicans* and *Streptococcus mutans*: in vitro study. Lasers Med Sci. 2019;34(1):129-37. doi: 10.1007/s10103-018-2622-6.
16. Costa AC, Rasteiro VM, Pereira CA, Rossoni RD, Junqueira JC, Jorge AO. The effects of rose Bengal- and erythrosine-mediated photodynamic therapy on *Candida albicans*. Mycoses. 2012;55(1):56-63. doi: 10.1111/j.1439-0507.2011.02042.x.
17. Dovigo LN, Carmello JC, de Souza Costa CA, Vergani CE, Brunetti IL, Bagnato VS, et al. Curcumin-mediated photodynamic inactivation of *Candida albicans* in a murine model of oral candidiasis. Med Mycol. 2013;51(3):243-51. doi: 10.3109/13693786.2012.714081.
18. Garcez AS, Ribeiro MS, Tegos GP, Núñez SC, Jorge AO, Hamblin MR. Antimicrobial photodynamic therapy combined with conventional endodontic treatment to eliminate root canal biofilm infection. Lasers Surg Med. 2007;39(1):59-66. doi: 10.1002/lsm.20415.
19. Azizi A, Amirzadeh Z, Rezai M, Lawaf S, Rahimi A. Effect of photodynamic therapy with two photosensitizers on *Candida albicans*. J Photochem Photobiol B. 2016;158:267-73. doi: 10.1016/j.jphotobiol.2016.02.027.
20. Du M, Xuan W, Zhen X, He L, Lan L, Yang S, et al. Antimicrobial photodynamic therapy for oral *Candida* infection in adult AIDS patients: a pilot clinical trial. Photodiagnosis Photodyn Ther. 2021;34:102310. doi: 10.1016/j.pdpdt.2021.102310.
21. Yamazaki H, Ohshima T, Tsubota Y, Yamaguchi H, Jayawardena JA, Nishimura Y. Microbicidal activities of low frequency atmospheric pressure plasma jets on oral pathogens. Dent Mater J. 2011;30(3):384-91. doi: 10.4012/dmj.2010-190.
22. Kostov KG, Borges AC, Koga-Ito CY, Nishime TM, Prysiazhnyi V, Honda RY. Inactivation of *Candida albicans* by cold atmospheric pressure plasma jet. IEEE Trans Plasma Sci IEEE Nucl Plasma Sci Soc. 2014;43(3):770-5. doi: 10.1109/tps.2014.2360645.
23. Sun Y, Yu S, Sun P, Wu H, Zhu W, Liu W, et al. Inactivation of *Candida* biofilms by non-thermal plasma and its enhancement for fungistatic effect of antifungal drugs. PLoS One. 2012;7(7):e40629. doi: 10.1371/journal.pone.0040629.
24. Fricke K, Koban I, Tresp H, Jablonowski L, Schröder K, Kramer A, et al. Atmospheric pressure plasma: a high-performance tool for the efficient removal of biofilms. PLoS One. 2012;7(8):e42539. doi: 10.1371/journal.pone.0042539.
25. Daliri F, Azizi A, Goudarzi M, Lawaf S, Rahimi A. In vitro comparison of the effect of photodynamic therapy with curcumin and methylene blue on *Candida albicans* colonies. Photodiagnosis Photodyn Ther. 2019;26:193-8. doi: 10.1016/j.pdpdt.2019.03.017.
26. Ebrahimi-Shaghaghi F, Noormohammadi Z, Atyabi SM, Razzaghi-Abyaneh M. Inhibitory effects of cold atmospheric plasma on the growth, virulence factors and HSP90 gene expression in *Candida albicans*. Arch Biochem Biophys. 2021;700:108772. doi: 10.1016/j.abb.2021.108772.
27. Quindós G, Gil-Alonso S, Marcos-Arias C, Sevillano E, Mateo E, Jauregizar N, et al. Therapeutic tools for oral candidiasis: current and new antifungal drugs. Med Oral Patol Oral Cir Bucal. 2019;24(2):e172-80. doi: 10.4317/medoral.22978.
28. Borges AC, Castaldelli Nishime TM, Kostov KG, de Morais Gouvêa Lima G, Lacerda Gontijo AV, de Carvalho JN, et al. Cold atmospheric pressure plasma jet modulates *Candida albicans* virulence traits. Clin Plasma Med. 2017;7-8:9-15. doi: 10.1016/j.cpme.2017.06.002.
29. Wanachantararak P, Suanpoot P, Nisoa M. Inhibitory activity of cold atmospheric plasma on *Candida albicans*. Walailak J Sci Technol. 2019;16(6):401-8. doi: 10.48048/wjst.2019.4811.
30. Panngom K, Baik KY, Nam MK, Han JH, Rhim H, Choi EH. Preferential killing of human lung cancer cell lines with mitochondrial dysfunction by nonthermal dielectric barrier discharge plasma. Cell Death Dis. 2013;4(5):e642. doi: 10.1038/cddis.2013.168.
31. Misra NN, Yadav B, Roopesh MS, Jo C. Cold plasma for effective fungal and mycotoxin control in foods: mechanisms, inactivation effects, and applications. Compr Rev Food Sci Food Saf. 2019;18(1):106-20. doi: 10.1111/1541-4337.12398.
32. He M, Duan J, Xu J, Ma M, Chai B, He G, et al. *Candida albicans* biofilm inactivated by cold plasma treatment in vitro and in vivo. Plasma Process Polym. 2020;17(4):1900068. doi: 10.1002/ppap.201900068.
33. Ma J, Shi H, Sun H, Li J, Bai Y. Antifungal effect of photodynamic therapy mediated by curcumin on *Candida albicans* biofilms in vitro. Photodiagnosis Photodyn Ther. 2019;27:280-7. doi: 10.1016/j.pdpdt.2019.06.015.
34. Marques Meccatti V, de Souza Moura L, Guerra Pinto J, Ferreira-Strixino J, Abu Hasna A, Alves Figueiredo-Godoi LM, et al. *Curcuma longa* L. extract and photodynamic therapy are effective against *Candida* spp. and do not show toxicity in vivo.

Int J Dent. 2022;2022:5837864. doi: 10.1155/2022/5837864.
35. Heidari L, Shirani AM, Amini S, Sanjari Pour S. Evaluation of photodynamic therapy with 660 nm diode laser and different concentrations of methylene blue on *Candida albicans* growth on denture (laboratory study). J Res Dent Sci. 2022;19(3):203-9. doi: 10.52547/jrds.19.3.203. [Persian].
36. Mardani M, Kamrani O. Effectiveness of antimicrobial photodynamic therapy with indocyanine green against the standard and fluconazole-resistant *Candida albicans*. Lasers Med Sci. 2021;36(9):1971-7. doi: 10.1007/s10103-021-03389-9.
37. Wiench R, Skaba D, Stefanik N, Kępa M, Gilowski Ł, Cieślar G, et al. Assessment of sensitivity of selected *Candida* strains on antimicrobial photodynamic therapy using diode laser 635 nm and toluidine blue - in vitro research. Photodiagnosis Photodyn Ther. 2019;27:241-7. doi: 10.1016/j.pdpdt.2019.06.007.
38. Seyedmousavi S, Hashemi SJ, Rezaie S, Fateh M, Esmaeeli Djavid G, Zibafar E, et al. Effects of low-level laser irradiation on the pathogenicity of *Candida albicans*: in vitro and in vivo study. Photomed Laser Surg. 2014;32(6):322-9. doi: 10.1089/pho.2012.3387.
39. Souza RC, Junqueira JC, Rossoni RD, Pereira CA, Munin E, Jorge AO. Comparison of the photodynamic fungicidal efficacy of methylene blue, toluidine blue, malachite green and low-power laser irradiation alone against *Candida albicans*. Lasers Med Sci. 2010;25(3):385-9. doi: 10.1007/s10103-009-0706-z.
40. Queiroga AS, Trajano VN, Lima EO, Ferreira AF, Queiroga AS, Limeira FA Jr. In vitro photodynamic inactivation of *Candida* spp. by different doses of low power laser light. Photodiagnosis Photodyn Ther. 2011;8(4):332-6. doi: 10.1016/j.pdpdt.2011.08.005.
41. Najafi S, Sheykhbahaei N, Khayamzadeh M, Gholizadeh N. The effect of low-level laser on number of *Candida albicans* colonies in-vitro: a new finding. BMC Oral Health. 2019;19(1):104. doi: 10.1186/s12903-019-0814-5.
42. Movaghari Pour A, Sheikh Fathollahi M, Poor Zamani M, Abedini S, Jamali Z. Comparison of anti-fungal effect of *Origanum vulgare* extract versus nystatin on *Candida albicans*; an in vitro study. J Mashhad Dent Sch. 2018;42(3):271-7. doi: 10.22038/jmds.2018.11470. [Persian].
43. Nozari S, Haydari Kohan F, Ahmadi F, Asadi M, Fallahi F, Ghasemi Z, Falahati M. Comparision of antifungal effect of Nystatin alone and in combination with nanosilver particles against *Candida* species isolated from chronic candidal vaginitis. Razi J Med Sci. 2013;19(104):60-6.
44. de Lacerda Gontijo SM, Gomes AD, Gala-García A, Sinisterra RD, Cortés ME. Evaluation of antimicrobial activity and cell viability of *Aloe vera* sponges. Electron J Biotechnol. 2013;16(1):1-10. doi: 10.2225/vol16-issue1-fulltext-2.