

Effects of near-infrared laser radiation on the survival and inflammatory potential of *Candida* spp. involved in the pathogenesis of chemotherapy-induced oral mucositis

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Abstract *Candida* spp. usually colonize ulcerative lesions of atrophic mucosa in patients with chemotherapy-induced oral mucositis inducing severe inflammation. The spread of antifungal-resistant strains strongly encouraged the search of complementary or alternative therapeutic strategies to cure inflamed mucosa. In this paper, we studied the effects of a near-infrared (NIR) laser system with dual-wavelength emission (808 nm+904 nm) on the survival and inflammatory potential of *C. albicans*, *C. glabrata*, and *C. parapsilosis*. Laser treatment was performed with a Multiwave Locked System laser. Survival and apoptosis of fungal strains were evaluated by colony-forming units (CFU) counting and annexin V staining. Cytokine production was evaluated by ImmunoPlex array. Laser treatment significantly affected the survival of *Candida* spp. by inducing apoptosis and induced a lower production of inflammatory cytokines by dendritic cells compared to untreated fungi. No differences in the survival and inflammatory potential were recorded in treated or untreated

Saccharomyces cerevisiae cells, used as the control non-pathogenic microorganism. Laser treatment altered the survival and inflammatory potential of pathogenic *Candida* spp. These data provide experimental support to the use of NIR laser radiation as a co-adjuvant of antifungal therapy in patients with oral mucositis (OM) complicated by *Candida* infections.

Introduction

Chemotherapy-induced oral mucositis (OM) are frequently complicated by infections with *Candida* spp. [1, 2], in particular with *C. albicans*. Recently, however, *C. glabrata*, *C. tropicalis*, and *C. parapsilosis* were also shown to be involved in the clinical course of OM [3]. The ulcerative lesions of atrophic mucosa represent an ecological niche optimal for the growth of pathogenic microorganisms, including opportunistic fungi, and the infection-induced inflammatory process significantly worsens the clinical symptomatology, leading to the appearance of white spots over inflamed areas. Pain, mouth irritation, and a burning sensation might have a great impact on the patient's well-being, affecting taste and the ability to eat and/or speak. Antifungal therapy is the first choice of treatment and it is strongly recommended [4]. However, the spread of resistance to common chemotherapeutics (fluconazole) and the propensity of several fungal strains to form biofilms [2], representing an impermeable barrier to antifungal drugs, increase the difficulty in treating these patients. So, the search for alternative therapeutic strategies is highly encouraged.

Laser therapy, applied to the oropharyngeal area, was reported to be as effective in the prevention of OM and in the reduction of severity, pain, and duration of OM ulcers [5, 6].

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In these studies, treatments have been performed by using a wide range of sources, differing in power (class I–III lasers, with power lower than 0.5 W, and class IV lasers, with power higher than 0.5 W), wavelength [mostly in the red and near-infrared (NIR) bands], and emission (single and combined, continuous and pulsed mode). Although some studies reported an increase in the mortality of *C. albicans* in vitro [7–9], the molecular and cellular mechanisms underlying the effects of laser therapy on microorganisms and host tissues remain largely unknown. Recently, the effects of two treatment protocols differing in wavelength (λ 635 nm versus λ 970 nm) and energy density (0.375 J/cm² versus 375 J/cm²) have been studied and compared in an animal model of chemotherapy-induced OM. Both treatments were effective in curing OM lesions; however, a better healing and a marked decrease in inflammation was reported in subjects treated with the higher energy density, suggesting its potential clinical application. In this study, however, the treatment parameters have been chosen without considering the direct effect of laser treatment on infectious agents complicating the clinical course of OM. Furthermore, the authors did not discuss that, beside the energy density, the treatments differed also for the wavelength, which is an important parameter in determining the chromophores involved and the therapeutic effects [10].

In the present study, we hypothesize that, beside the homeostasis of host tissues, laser therapy may affect pathogen survival and host inflammatory response, and that the combination of these effects could improve the clinical course of OM. Therefore, prospecting the use of a dual-wavelength, NIR laser system in patients with OM, we explored the effects of such laser emission on the survival and inflammatory potential of those *Candida* spp. (*C. albicans*, *C. glabrata*, *C. parapsilosis*) which frequently colonize the injured and inflamed tissues in OM patients [1, 2, 11].

Materials and methods

Reagents

Annexin V was from Roche Diagnostics (Mannheim, Germany). pNiFty2-luc NF- κ B-inducible reporter plasmid, Zymosan, and lipopolysaccharide (LPS) were from InvivoGen (San Giuliano Milanese, Italy). RPMI 1640, penicillin/streptomycin (P/S), heat-inactivated fetal bovine serum (FBS), and DMEM 4.5 g/l glucose were from Celbio (Pero, Italy). Solid (YPD-A) and liquid (YPD) yeast culture media were from Sigma-Aldrich (Sigma-Aldrich Chemical Co., St. Louis, MO, USA). The Dual-Glo Luciferase Activity Kit was purchased from Promega (Madison, WI, USA). Human recombinant (hr) IL4 and hr GM-CSF were from R&D (R&D Systems, Minneapolis, MN, USA). The multiplex

immunoarray kit for cytokines assay was purchased from Millipore (Billerica, MA USA).

Cultures

Yeasts: *C. albicans* (SC53-14, ATCC MYA-2876), *C. parapsilosis* (YA4), *C. glabrata* (YUC23), and *Saccharomyces cerevisiae* (S288c) strains were grown in agar plates (YPD-A, bacteriological peptone 20 g/L, yeast extract 10 g/L, glucose 20 g/L, agar 15 g/L) at 28 °C for about 2 days. Fungal colonies were cultured in liquid broth medium (YPD, bacteriological peptone 20 g/L, yeast extract 10 g/L, glucose 20 g/L) at 28 °C for 18 h before the treatment.

Cells: HEK-293-hTLR4/CD14/MD2 and HEK-293-hTLR2 were stably transfected with pNiFty2-luc NF- κ B-inducible reporter plasmid by electroporation (355 V for 10 ms) and maintained in DMEM 4.5 g/L supplemented with 10 % FBS.

Laser treatment

The treatments were performed with a Multiwave Locked System (MLS) laser (Mphi, ASA Srl, Arcugnano, VI, Italy), a commercial laser source built in compliance with EC/EU rules, approved by the U.S. Food and Drug Administration (FDA), as previously reported [12]. The MLS is a class IV NIR laser with two synchronized sources (laser diodes). The two modules have different wavelengths, peak power, and emission mode. The first is the pulsed laser diode, emitting at 905 nm, with 25 W peak optical power; each pulse is composed of a pulse train (100 ns single pulse width, 90 kHz maximum frequency). The frequency of the pulse trains may be varied in the range 1–2000 Hz, thus varying the average power delivered to the tissue. The second laser diode (808 nm) may operate in continuous (power 1.1 W) or frequency mode (repetition rate 1–2000 Hz), 550 mW mean optical power output, duty ratio 50 % independently of the repetition rate. The two laser beams work simultaneously, synchronously, and the propagation axes are coincident.

For laser treatment, cells were harvested after 18 h of culture, washed twice in distilled water, and plated in triplicate in 24 well/plates at 10⁷ cells/ml. Plates were placed inside a holder to allow controlled scan treatment; they were aligned with the hand piece, sliding in an overlying track, at a distance of 2 cm from the bottom of the wells, so that the spot of the two superimposed laser beams, impinging perpendicular to the sample surface, had the same diameter as a well (13 mm). A single experienced operator performed all the treatments. The scan of the samples was performed manually by moving the hand piece with a speed of 5.6 cm/s, under the guide of a metronome. The treatment was administered in sterile conditions and included two exposures to laser

radiation, with a 1-h interval. The scan mode for treatment was chosen to mime treatment conditions often used in clinics.

Four treatment protocols differing in exposure time and total energy delivered were tested (Table 1).

Count of colony-forming units

Soon after treatment, 100 μ L from each fungal culture was diluted to 1:100,000 and plated in triplicate in YPD-A at 28 °C. Colony-forming units (CFU) were counted after 24 and 48 h. Untreated or heat-killed (95 °C for 15 min) fungal cells were used as controls.

Isolation and maturation of dendritic cells

To obtain monocyte-derived dendritic cells (MDDC), CD14+ cells, purified from the peripheral blood of healthy donors, were cultured in the presence of 20 ng/ml of hr IL4 and 50 ng/ml of hr GM-CSF (R&D) at 37 °C in a humidified chamber with 5 % CO₂ for 5 days [13]. Non-adherent cells were stimulated for 16 h with treated or untreated fungal strains.

Toll-like receptors (TLRs) assay

HEK-293-hTLR4/CD14/MD2 and HEK-293-hTLR2 cells, stably transfected with pNiFty2-luc plasmid, plated in DMEM/FBS in 96 wells at 2×10^5 /well and stimulated with 10^5 heat-killed (95 °C for 15 min), laser-treated (200 J, with 1500 Hz frequency), or untreated cells of *Candida* spp. (*C. albicans*, *C. glabrata*, *C. parapsilosis*) and of *S. cerevisiae*. Zymosan (100 μ g/ml) or LPS (50 ng/ml) were used as positive controls for TLR2 and TLR4, respectively. Luciferase activity was measured 8 h later by the Dual-Glo Luciferase Assay System (Promega).

Cytokines assay

IL1- β , IL6, IL12p70, and IL-23 concentrations in MDDC culture supernatants were determined by using the Milliplex kit and Bio-Plex apparatus, according to the manufacturer's recommendations.

Table 1 Frequency, time of exposure, and energy density used relative to a single exposure to laser radiation. Each treatment included two exposures of fungi to laser radiation, with a 1-h interval

Protocol	Frequency	Time	Energy density (J/cm ²)
P1	1500 Hz	1'15"	2
P2	1500 Hz	2'29"	3.5
P3	1500 Hz	4'58"	7
P4	1500 Hz	12'24"	18

Annexin V/PI labeling

C. albicans, *C. parapsilosis*, *C. glabrata*, and *S. cerevisiae* strains were cultured overnight in YPD at 28 °C. 100 μ l of culture were diluted in 4 ml of YPD for a further 4 h at 28 °C, washed, and split into three aliquots of 2×10^6 cells each. One aliquot was laser-treated (200 J, 1500 Hz). Untreated fungi or fungi treated with 175 mM acetic acid were used as controls. Fungi were treated with Zymolyase (2 μ g/ml in H₂O-P/S) at 37 °C for 30 min, washed, and stained for 20 min with 100 μ l of FITC-annexin V/PI mix. Cytofluorimetric analysis was performed by the Accuri instrument. Ten thousand events for each sample were acquired. Data were analyzed by CFlow Plus software.

Results

Effect of different laser treatments on yeast survival

C. albicans (SC53-14, ATCC MYA-2876) and *S. cerevisiae* S288c strains [14] were used as representatives of pathogenic and non-pathogenic yeasts, respectively, and treated with different laser irradiation protocols, as indicated in Table 1. Figure 1 shows that all the tested protocols were able to decrease the number of CFU of *C. albicans*, while *S. cerevisiae* cells were not responsive to the treatments. Regarding *C. albicans*, the maximum effect in terms of mortality rate was reached with treatment protocol P3 (7 J/cm²).

Thus, treatment protocol P3 was chosen for the following experiments.

Effect of laser treatment on different *Candida* spp.

It is known that, besides *C. albicans*, different *Candida* spp., such as *C. glabrata* and *C. parapsilosis*, even more frequently complicate the clinical course of OM [11]. We, thus, wondered whether laser treatment (protocol P3) could affect the survival of these other *Candida* spp., as observed for *C. albicans*.

C. albicans, *C. parapsilosis*, *C. glabrata*, and *S. cerevisiae* cells were cultured and treated as reported above. Figure 2a shows that this treatment significantly reduced (40 %) the number of CFU for *C. albicans* and *C. glabrata* and, to a lesser extent (27 %), that of *C. parapsilosis*; again, *S. cerevisiae* was totally insensitive to laser radiation.

Effect of laser treatment on apoptosis

In order to understand the mechanism underlying the reduction in the CFU number, we investigated whether laser treatment induced apoptosis of fungal strains, evaluating by cytofluorimetric analysis the percentage of annexin V-

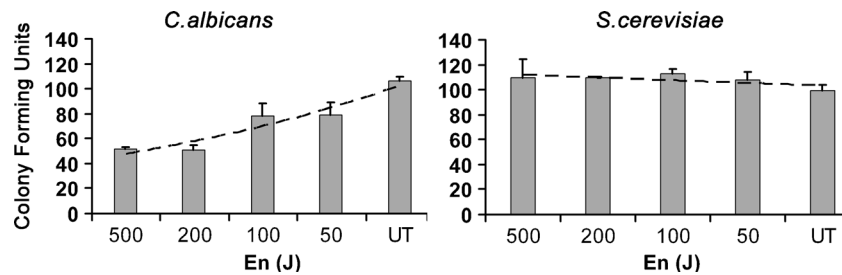


Fig. 1 *Candida albicans* and *Saccharomyces cerevisiae* strains were grown in YPD supplemented with 1 % yeast extract, 2 % peptone, and 2 % glucose for 18 h, plated in 24-well plates at 10^7 /ml, and subjected to two exposures to laser radiation, with a 1-h interval. Four protocols differing in energy doses were tested. After treatment, 100 μ l of $1:10^5$

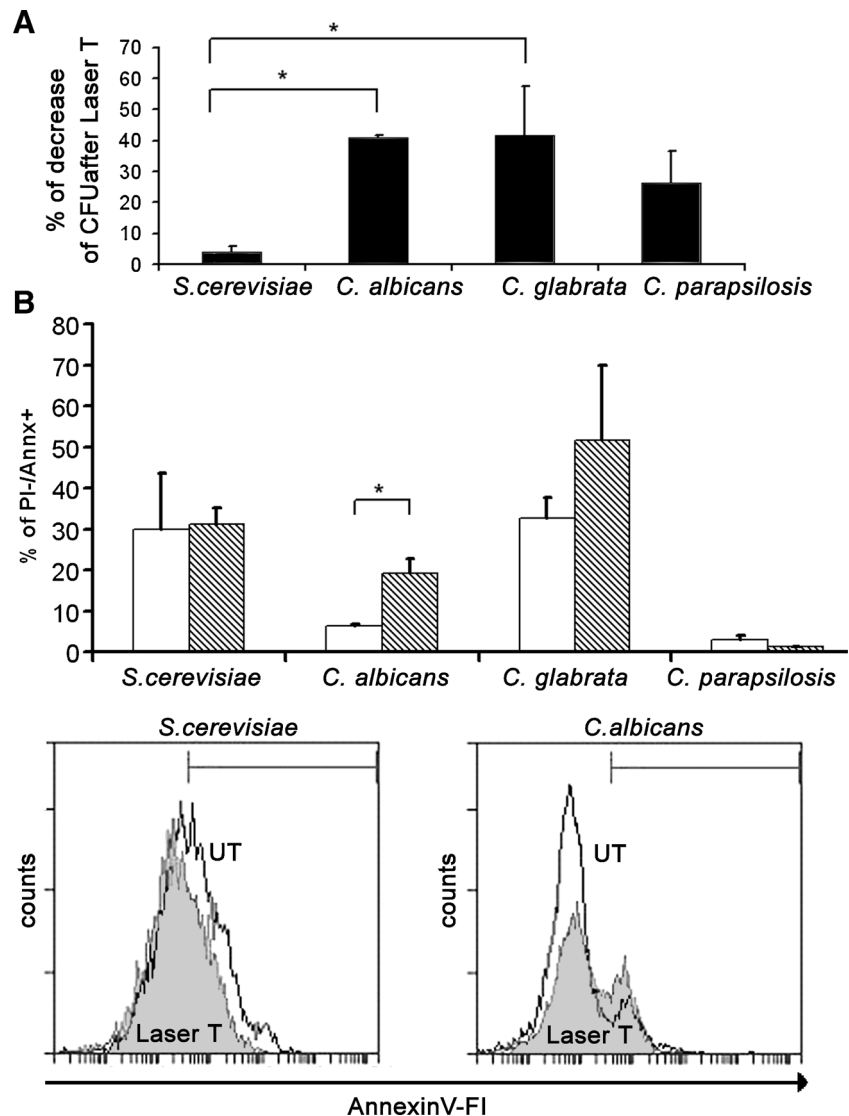
cellular dilutions were plated in triplicate in YPD agar and incubated at 28 °C. Data are shown as the mean count of colony-forming units (CFU) determined 2 days after treatment. The results from five different experiments [mean CFU \pm standard error (SE)] are reported

positive cells. Figure 2b shows that laser radiation significantly increased apoptotic death in *C. albicans* and *C. glabrata* strains, while it leaves unaffected the survival of *C. parapsilosis*. Furthermore, laser treatment did not induce apoptosis in non-pathogenic *S. cerevisiae* cells.

Effect of laser treatment on the inflammatory potential of fungal cells

Infections with *Candida* spp. always induce the production of proinflammatory cytokines and chemokines by cells of innate

Fig. 2 *C. albicans*, *C. parapsilosis*, *C. glabrata*, and *S. cerevisiae* cells were grown in YPD supplemented with 1 % yeast extract, 2 % peptone, and 2 % glucose for 18 h, plated in 24-well plates at 10^7 /ml, and subjected to laser treatment (two exposures separated by a 1-h interval, energy density per exposure 7 J/cm², 1500 Hz frequency). The results are shown as percentage of decrease in CFU numbers detected 2 days after laser treatment. The data (mean \pm SE) from 11 different experiments are shown. Statistical analysis was performed by Student's *t*-test. $p \leq 0.05$ was considered significant



immunity, which recruit inflammatory cells at the site of *Candida* invasion. Since laser treatment could induce biochemical changes in fungal recognition patterns, we investigated how fungal cells stimulate the production of inflammatory cytokines by MDDC before and after the treatment.

MDDC were cultured for 24 h with laser-treated or untreated *Candida* or *S. cerevisiae* strains. Heat-killed cells and LPS (50 ng/ml) were used as positive controls. At the end of the incubation, conditioned media were collected and the production of inflammatory cytokines was measured through multiplex immunoarray. Figure 3a shows that *Candida* spp. and *S. cerevisiae* were able to induce the production of the inflammatory cytokines IL-1 β and IL-6. However, relevant differences in the induction of cytokine production by live, untreated cells from these species were recorded, with *C. albicans* inducing the lowest amounts, while *S. cerevisiae* always induced the maximal production of IL-1 β and IL-6. As expected, the killed forms of the fungi (positive controls) induced a

pattern of cytokine production different from that of their respective live (untreated) forms, likely reflecting changes in the exposure of cell wall components as a consequence of heat killing. Figure 3b shows that laser treatment significantly modulated the ability of *C. glabrata* and *C. parapsilosis* to induce IL-1 β production by MDDC and further inhibited that of *C. albicans* cells. The production of IL-6 by MDDC was also decreased following the exposure of MDDC to irradiated pathogenic *Candida* spp. In contrast, the exposure to laser radiation left unaffected the ability of *S. cerevisiae* cells to induce IL-1 β and IL-6 production by MDDC.

Beside a species-dependent variability in inducing the production of inflammatory cytokines by cells of innate immunity, these data indicate that laser treatment could affect the inflammatory potential of pathogenic *Candida* spp., while leaving unaffected that of harmless yeast.

We also measured in the same culture supernatants the production of IL-12p70 and IL-23, two cytokines

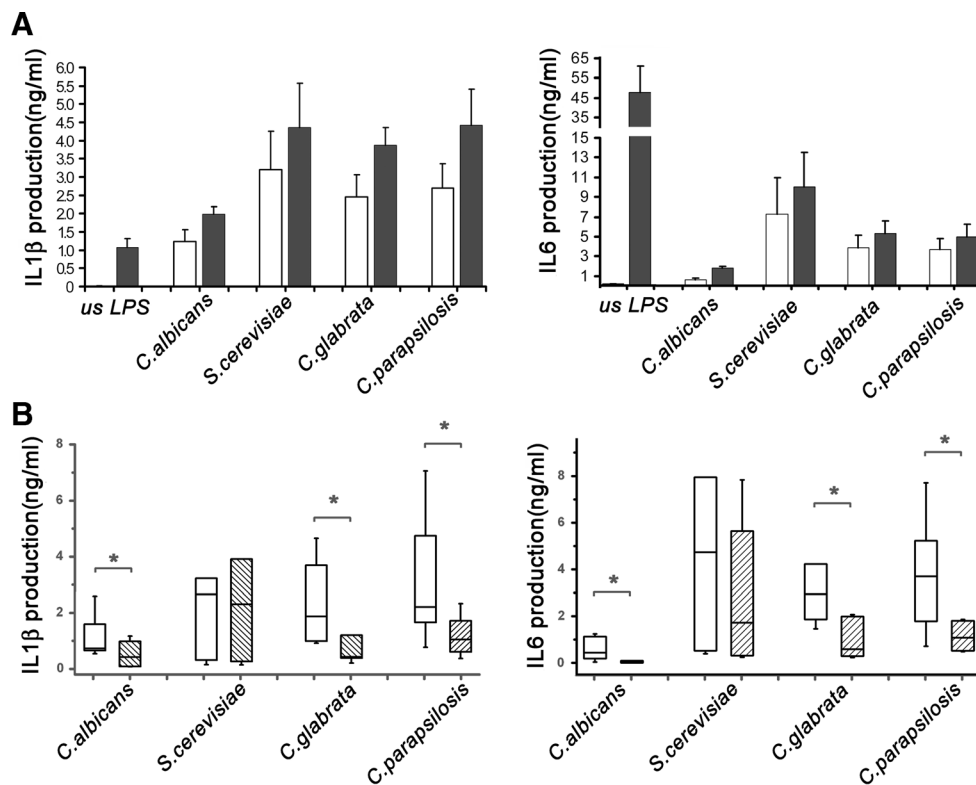


Fig. 3 *C. albicans*, *C. parapsilosis*, *C. glabrata*, and *S. cerevisiae* cells were grown in YPD supplemented with 1 % yeast extract, 2 % peptone, and 2 % glucose for 18 h, plated in 24-well plates at 10^7 /ml, and subjected to laser treatment (two exposures separated by a 1-h interval, energy density per exposure 7 J/cm², 1500 Hz frequency). Human monocyte-derived dendritic cells (MDDC), obtained through the differentiation of CD14⁺ circulating monocytes in the presence of IL-4 and GM-CSF, were cultured for 24 h with laser-treated or untreated *C. albicans*, *C. parapsilosis*, *C. glabrata*, and *S. cerevisiae* strains at the ratio of 1:10. Heat-killed (95 °C for 15 min) fungi and lipopolysaccharide (LPS; 50 ng/ml) were used as positive controls. At the end of incubation, conditioned media were collected and the production of

inflammatory cytokines measured through multiplex immunoarray. **a** Production of IL-1 β and IL-6 by dendritic cells stimulated with untreated (white columns) or heat-killed (black columns) *Candida* spp. *S. cerevisiae*. The results are shown as the mean cytokine production \pm SE of six different experiments. **b** Box-and-whisker plots show IL1 and IL6 production by MDDC cultured with laser-treated (gray boxes) or untreated (white boxes) *C. albicans*, *C. parapsilosis*, *C. glabrata*, and *S. cerevisiae* strains. The data are representative of six different experiments. The boxes extend from the 25th percentile to the 75th percentile, with a horizontal line at the median (50th percentile). The whiskers indicate outliers. Statistical analysis was performed by the non-parametric Mann–Whitney test; $p \leq 0.05$ was considered significant

involved in T-helper (TH)-1 and TH-17 differentiation response, respectively [15]. We could not observe significant differences in the production of these cytokines by MDDC exposed to treated and untreated different fungal strains, suggesting that the development of the immune specific response could not be affected at least in immunocompetent hosts.

Effect of laser treatment on the ability of fungal cells to activate the TLR2 and TLR4 pathways

In MDDC, recognition of *C. albicans* at the level of the cell membrane is mediated by TLRs and C-type lectin receptors (CLRs). TLR4 mainly induces proinflammatory signals, while TLR2 induces a moderate proinflammatory response and a strong IL-10 production [16].

We, thus, investigated whether the exposure to laser radiation impaired the ability of *Candida* spp. and/or *S. cerevisiae* to activate TLR4 and TLR2 in specific assays, using HEK293 cells transfected with huTLR4 or huTLR2. Figure 4 shows that untreated *Candida* and *S. cerevisiae* fungal cells induced a slight stimulation of TLR4 activity. Laser treatment of fungal cells always decreases the ability to stimulate TLR4, but the differences between treated and untreated cells reached statistical significance only using *C. albicans* cells. In contrast, no differences were detected in TLR2 stimulation by treated fungal cells of any species compared to untreated cells (data not shown).

Discussion

Laser therapy, performed by using different laser sources and treatment parameters (low- or high-power laser sources, red or NIR wavelengths, continuous or pulsed modality), has been applied to the oropharyngeal area for the prevention and treatment of OM. The efficacy of treatment was reported in different studies with differences depending on sources and

treatment parameters; laser therapy has been shown to be effective in the prevention of OM and in the reduction of the severity, pain, and duration of OM ulcers [5, 6], even in those induced by chemotherapy [10]. However, the direct effects of laser therapy on pathogens colonizing the atrophic mucosae have not been detailed and clinical studies do not consider this point in the choice of protocols.

This paper describes the effects of a dual-wavelength, NIR laser source (MLS laser) on the survival and inflammatory potential of *C. albicans*, *C. glabrata*, and *C. parapsilosis*, representing the most frequent pathogens complicating the clinical course of chemotherapy- or radiotherapy-induced OM. Our data clearly show that the selected treatment parameters (P3) significantly affected the survival of pathogenic *Candida* spp., while they left unaffected that of *S. cerevisiae* cells. A further increase in energy density (P4) did not correspond to an increase of effect (see Fig. 1).

The generation of reactive oxygen species (ROS), resulting from the absorption of laser radiation at the mitochondrial level, has been proposed as a major molecular mechanism underlying the photoinactivating action of laser therapy on microbial species, including *C. albicans* and *Staphylococcus* [9]. Indeed, increased ROS generation might be responsible for the induction of apoptosis observed in our experiments. This mechanism, however, could not explain the different outcomes in terms of survival activity (or residual CFU activity) induced by laser treatment in *Candida* spp. and *S. cerevisiae*, since common core mechanisms for oxidative stress response are shared by these fungal species [17–19]. In our opinion, the different sensitivities of fungal species to laser treatment could, at least in part, reside in structural and physicochemical features involved in the survival as well as in the virulence of pathogenic *Candida* spp. The fungal wall composition, easily to be involved in treatment sensitivity, varies between different fungal species and even among their strains. It also varies between filamentous fungus and

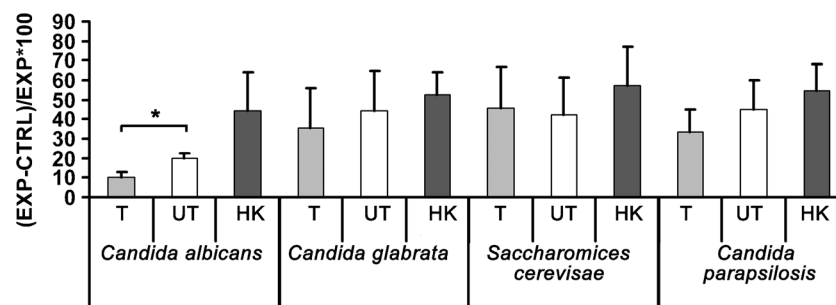


Fig. 4 HEK-293-hTLR4/CD14/MD2 cells stably transfected with pNiFty2-luc NF-kB-inducible reporter plasmid were plated in 96 wells (2×10^5 /well) and stimulated with 10^5 heat-killed (HK, black columns), laser-treated (gray columns), or untreated (white columns) cells of *Candida* spp. (*C. albicans*, *C. glabrata*, *C. parapsilosis*) and of

S. cerevisiae for 8 h. Lipopolysaccharide (LPS) was used as the positive control. The data are shown as the TLR4 stimulation index calculated as the luciferase activity of stimulated HEK-293 cells by treated versus untreated yeast cells. Statistical analysis was performed by Student's *t*-test and $p \leq 0.05$ was considered significant

yeast. The yeast-to-hypha transition, which does not occur in *S. cerevisiae*, leads to a major increase in the distribution, adhesion, unfolding, and extension of adhesins and their associated mannans [20, 21], and the process is usually accompanied by an increase in the exposure of mannosylation [20, 21]. It is reasonable that laser treatment affects the composition of structures which are more exposed during the transition from yeast to hyphae cells. Supporting this hypothesis are preliminary data, obtained in our laboratory, showing that cultures of *Candida* spp. in Sabouraud's medium, which strongly prevents hyphae formation, are not sensitive to laser radiation in terms of survival impairment. Furthermore, clinical isolates of *C. albicans* with different capacities to form hyphae also differ in sensitivity to laser treatment (data not shown).

Beside the effects on survival of *Candida* spp., our data also show how laser treatment affects the inflammatory potential of *Candida* spp., while it leaves unaffected that of *S. cerevisiae*. According to different reports [22], we found relevant differences in the production of two inflammatory cytokines (IL-1 β and IL-6) by human mature MDDC exposed to live fungal strains. In this system, *C. albicans* induced the lowest amounts of IL-1 β and IL6, while *S. cerevisiae* induced much higher production of these cytokines. The fungal cell wall structure is composed of chitin, β -glucans, and mannoproteins, proteins with mannans covalently attached via ester linkages (O-linked mannans) or by amide bonds (N-linked mannans). Cells of innate immunity, including conventional mature MDDC, recognize fungal pathogen-associated molecular patterns (PAMPs) through TLRs and CLR s [23]. Since the hyphae formation process is accompanied by an increase in the exposure of mannans, different pattern recognition receptors (PRRs) could be engaged [20, 21]. Thus, once more, the dimorphic state of *Candida* spp., but not that of *S. cerevisiae*, could account for differences in cytokine production by mature MDDC. Recently, however, sugar moieties as β -1,2-mannosides of N-linked mannan, present in *C. albicans* but not in *S. cerevisiae* [24], have been reported as being responsible for the lower inflammatory potential of *C. albicans* compared to *S. cerevisiae*. *C. albicans* mutants lacking the N-linked mannan β -1,2-mannosides induced much higher production of inflammatory cytokines compared to wild-type strains [25]. These structures could account for the lower inflammatory potential of *Candida* spp. compared to *S. cerevisiae* in a manner not dependent on morphological status [25]. Since these premises, we also take into account the possibility that N-linked mannan β -1,2-mannosides might be a target of laser treatment; indeed, MDDC cultured with laser-treated *Candida* spp. further

decreased their cytokine production compared to untreated *Candida* strains and to *S. cerevisiae*. This hypothesis, however, needs to be investigated further. Laser treatment slightly decreased the ability of *Candida* spp. to activate TLR4 pathways, but the irradiation did not affect TLR4 activation by *S. cerevisiae*. The TLR2 activation was unaffected by laser radiation, suggesting, once more, that laser effects could not involve deeper layers of the fungal wall, but, rather, the more external ones.

In summary, limited to source, parameters, and mode of irradiation evaluated in this study, laser treatment was able to induce apoptosis in *Candida* spp. and impair fungal cell survival. Treatment-induced changes in external cell wall structures, as well as changes in membrane permeability and ROS accumulation, could cause apoptosis of fungal cells, thus limiting the release of proinflammatory molecules and increasing the possibility of being phagocytized by macrophages and MDDC.

Furthermore, while laser treatment significantly affected the production of IL-1 β and IL-6, it did not modify the production of IL-23 and IL-12, suggesting that TH-17 and TH-1 response needed for the clearance of pathogens [26] could proceed normally, at least in immunocompetent hosts.

Thus, we concluded that NIR laser treatment, performed with the source and parameters proposed here, was able to modify the survival and inflammatory potential of pathogenic *Candida* spp. It is conceivable that these effects are attributable, at least in part, to structural modifications of N- and O-linked mannans of the fungal cell wall. This hypothesis will be verified in future studies.

On the basis of the presented results, laser treatment could be helpful as an alternative to, or as a co-adjuvant of, antibiotic therapy in immunocompromised hosts with OM complicated with *Candida* infections. The combined effect of reduced fungal cell survival and inflammation could help to decrease the severity of symptoms and the duration of OM ulcers. Furthermore, since mannose-rich structures, and cell surface mannoproteins in particular, are also involved in mediating biofilm formation in *Candida* infection, the proposed treatment could also be helpful in *Candida* infections of different anatomic districts.

However, further studies are needed to better elucidate (i) how laser radiation affects fungal cell survival and what are the chromophores involved and (ii) how laser radiation affects the response/homeostasis of host tissues, i.e., behavior of immune cells, evolution of inflammation, and wound healing in diseased tissue. Indeed, it is known that laser radiation promotes wound healing [27–29]. Moreover, applying the same NIR laser source used in the present study (that is, the same wavelengths

and emission mode), we have recently demonstrated the overexpression of NLRP10, a protein involved in the activation of anti-inflammatory pathway, by animal myoblast cells [12, 30, 31], suggesting that such mechanisms can contribute to the overall therapeutic efficacy.

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Conflict of interest The authors declare no conflict of interest.

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