



Antimicrobial photodynamic therapy reduces adhesion capacity and biofilm formation of *Candida albicans* from induced oral candidiasis in mice

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ABSTRACT

Background: Antimicrobial photodynamic therapy (aPDT) has been considered an alternative therapeutic modality for the treatment of *Candida* infections. However, most studies are focused mainly on microorganism's inactivation efficiency. Here, we evaluated the efficacy of aPDT mediated by chloro-aluminum phthalocyanine encapsulated in cationic nanoemulsions (CIAIP-NE) to treat oral candidiasis in vivo and its effect on the adhesion and biofilm formation of *Candida albicans*.

Methods: For this, mice were immunosuppressed and inoculated with *C. albicans* to produce oral candidiasis. aPDT and Nystatin were applied for 5 successive sessions. Next, the microbiological evaluation was determined (CFU/ml) and the analyses of virulence factors (adhesion capacity and biofilm formation) were performed. Data were analyzed by Two-way ANOVA ($\alpha = 0.05$).

Results: aPDT was as effective as Nystatin reducing 1.4 and 2.0 log₁₀ of the cell viability ($p \leq 0.0001$), respectively. Both treatments reduced the adhesion capacity and biofilm formation of *C. albicans* ($p \leq 0.0001$).

Conclusion: CIAIP-NE-mediated aPDT was effective in reducing the virulence factors of *C. albicans* and also to treat induced oral candidiasis in mice.

1. Introduction

Oral candidiasis (OC), considered the most common human fungal infection [1], is caused by an overgrowth of *Candida* spp., in particular, *Candida albicans*, that has been isolated from the oral cavity of up to 75% of the population [2]. The incidence of this fungal infection depends on the age of the patients and convenient predisposing local/systemic factors, such as, poor oral hygiene, dentures, impaired salivary gland function, diabetes mellitus, prolonged exposure to broad spectrum antibiotics, organ transplant patients, and patients treated with immunosuppressive therapy [3]. In immunosuppressed individuals, OC can spread to the pharynx and/or the esophagus, or blood stream, increasing the rates of morbidity and mortality [4].

Currently, the treatment is based on the correction of predisposing

local factors and the use of topic or systemic azole antifungal drugs (fluconazole, itraconazole, voriconazole, posaconazole) [5], echinocandins (caspofungin, micafungin, anidulafungin) and amphotericin B [6,7]. However, the recurrence of infection and the resistance to these medicaments are often reported [6,7] and they are associated with the biofilm life style of *C. albicans* [8,9]. Biofilms have been described as microbial communities of adherent cells wrapped by an extracellular polymeric matrix, which allows the survival of fungal cells under hostile conditions [10]. The cells from *Candida* biofilms may release and spread throughout the host and colonize new sites of infection [11]. The pathogenesis of *Candida* infections seems to be mediated by some virulence factors, which permits the interaction of the microorganisms with the host cells promoting cellular damage [12,13]. Among all of virulence factors observed in *Candida* species, the adhesion and

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formation of biofilm are of great importance [13]. The adhesion of fungal cells to human tissues and to abiotic substrates, such as prostheses and catheters and, followed by biofilm formation on these surfaces, may be mediated by cell wall proteins through various chemical interactions with surface molecules [11]. Moreover, the failure in the treatment of OC is related to the complex physical structure of the biofilms, which difficult the penetration and action of the antifungal agents [5]. For this reason, there is an emerging interest in the development of new strategies to treat OC. One such approach is Anti-microbial Photodynamic Therapy (aPDT).

aPDT is based on the association of three components: a photosensitizer agent (PS), a visible light with specific wavelength and oxygen. The PS is excited by the light and, in the presence of oxygen, this reaction generates singlet oxygen and other reactive oxygen species (ROS), which are the main responsible to cause the cell damage by oxidative stress [14]. ROS interact with the cell constituents (proteins, membrane lipids and nucleic acids) by different pathways [15]. Chloroaluminum phthalocyanine (ClAlP) is a second-generation photosensitizer, with properties that facilitates its use in aPDT due to the high production quantities of singlet oxygen [16]. Recently, an *in vivo* study showed that aPDT mediated by ClAlP in cationic nanoemulsion (NE) was effective in the inactivation of *C. albicans* recovered from the oral cavity of immunosuppressed mice with OC, after one single application of the therapy [17]. While *in vitro* studies represent some aspects of OC, experimental animal models are more closely correlated to clinical situations and are essential to fully understand the pathogenesis of this disease and to evaluate new strategies of treatment. Recently, a reliable mouse model of OC was described using 5 sessions of aPDT to treat this infection [18]. By combining this animal model with aPDT treatment and the analyses of virulence factors, the present investigation aimed to contribute to *in vivo* aPDT studies by reporting the use of ClAlP-NE for the treatment of oral candidosis, and its relationship with the virulence factors (adhesion and biofilm formation) of *C. albicans*.

2. Materials and methods

2.1. Photosensitizer agent and parameters of irradiation

The photosensitizer employed here was ClAlP entrapped in NE (handled from the Center of Nanotechnology and Tissue Engineers, Photobiology and Photomedicine research Group - USP Ribeirão Preto, Brazil) [19], at a concentration of 31.7 μM [17]. ClAlP-NE was excited by the red LED equipment at 660 nm (LXHL-PR09, Luxeon® III Emitter, Lumileds Lighting, San Jose, CA, USA) homogeneously scattered, with a constant power output delivery of 44.6 mW/cm^2 . It was applied a dose of light equivalent to 100 J/cm^2 .

2.2. Induction of oral candidiasis

The present study was permitted by the Ethics Committee on Animal Use (CEUA) of School of Dentistry, Araraquara, UNESP (Permit Number: 24/2012), and it was conducted in agreement with the national (CONCEA –<http://concea.mct.gov.br>) and institutional laws of the same University.

The amount of 126 female Swiss mice was placed in cages housing in accordance with the experimental group that they belonged. They were kept in a room with light (12 h of lightness and 12 h of darkness) and temperature controlled ($23 \pm 2^\circ\text{C}$) and with free access to food and water.

The strain of *C. albicans* ATCC 90028 (Rockville, MD USA) was used to produce OC in mice, which was frozen at -80°C . The strain was reactivated and standardized suspensions were obtained at the concentration of 10^7 CFU/mL in RPMI 1640 medium [18].

The methodology used to produce the experimental oral candidiasis in mice, was previously described by Takakura et al. [20], and reproduced by our group [17,18], with some modifications. Briefly, on

day 1, animals were immunosuppressed with subcutaneous injections of prednisolone (100 mg/kg body weight). On day 2, animals received an intramuscular injection of chlorpromazine hydrochloride (Santa Paula Pharmacy, Araraquara, SP, Brazil) (0.1 mL of 2 mg/mL) and *Candida* inoculation, which was performed by swabbing the cotton-pad (Cotton baby, Higie-Plus Cotton baby Ind. Com., Ltd, San Jose, SC, Brazil) immersed in the *Candida* suspension (10^7 CFU/mL) on the tongue of all animals [17,18].

In accordance with the period of time evaluated (24 h and 7 days after treatment), animals received complementary subcutaneous injections of prednisolone to maintain the infection, which was performed on days 5, 9 and 13.

2.3. Therapies and microbiological analyses

On days 7 to 11, animals were submitted to the treatments, once a day, according to the experimental groups: P + L + group, constituted of animals treated with aPDT (31.7 μM of ClAlP-NE was applied on the dorsum of the tongue and they were kept in the dark for 20 minutes, pre-irradiation time, and the tongues were illuminated with a light dose equivalent to 100 J/cm^2) [17]; P + L- and P-L + groups, composed by animals that received the PS or light only, respectively; the positive control group or NYS group, constituted of animals treated with Nystatin oral suspension (100,000 IU). Animals from P-L- group (negative treatment control group) received *C. albicans* inoculation, but did not receive any treatment, and the NC group (negative control of infection) was composed by healthy animals. The number of animals used in each group was 12, excepted for the NC group, which consisted of 3 animals. Prior the treatments, mice were anesthetized with an intraperitoneal injection of ketamine and xylazine (100 mg/kg and 10 mg/kg, respectively - National Pharmaceutical Chemistry Union S/A, Embu, SP, Brazil, and Veterinary JA Ltda., Sponsor Paulista, SP, Brazil).

The microbiological analyses were performed 24 h and 7 days after the treatments, by recovering *C. albicans* from the dorsum of the tongue of mice using a sterile swab. The swabs were then vortexed in 1 mL of saline solution, which was submitted to serial dilutions, plated onto Sabouraud Dextrose Agar (SDA) medium with chloramphenicol and incubated for 48 h at 37°C . Then, the CFU/ml values were determined. After microbiological analyses, 800 μL of the remained saline solution containing *C. albicans* cells collected from the tongue of animals were frozen at -20°C until the moment of use for posterior analyses of virulence factors.

2.4. Evaluation of adhesion capacity and biofilm formation of *C. albicans* to abiotic surface

To evaluate the effect of the treatments on the adhesion of *C. albicans* to abiotic surface, the following methods were performed: determination of the number of cells adhered by colony forming units (CFU/mL) and analysis of the cellular metabolism by means of XTT assay [21].

To avoid that the results of adhesion capacity and biofilm formation achieved in the present investigation were influenced by the lower number of cells recovery from the tongue of mice after the treatments, the cell concentration observed in the group that demonstrated the highest reduction in the CFU/mL after microbiological analysis (NYS treatment) were chosen to standardize the cell concentration of all experimental groups. For this purpose, in all groups of treatments an aliquot of 800 μL of *C. albicans* recovered from the tongue lesion of mice were centrifuged at 5.000 g for 7 min, washed twice with 1 mL of PBS, resuspended in 1 mL of PBS and the cell concentration was adjusted in a spectrophotometer with an optical density (OD) of 540 nm and an absorbance of 0.2 (10^3 CFU/mL – concentration observed in the NYS group).

Then, the standardized suspension was transferred to a 96- well microtiter plate, and cells were submitted to the procedures of adhesion

to the bottom of the plate (adhesion phase) for 90 min at 37 °C [22]. After adhesion, *C. albicans* cells were detached from the bottom of the wells with a micropipette for dissociation. For cell quantification, serial dilution (10^0 to 10^{-3}) was performed, the cells were seeded in SDA culture medium, incubated at 37 °C for 48 h, and the CFU/mL values were determined.

With regard to the XTT assay, the attached cells were washed with 200 μ L of PBS, then, 200 μ L of XTT solution [23] were inserted into each well, incubated at 37 °C for 3 h in the dark. After this period, 100 μ L of the XTT reaction were transferred to another 96-well microtiter-plate and the colorimetric analysis was measured at 492 nm in spectrophotometer (Thermo Plate / TP Reader) [23].

For the biofilm formation, the same steps described above for culturing *C. albicans* on the wells were performed until the adhesion phase. Then, the wells were washed twice with PBS, an aliquot of 200 μ L of RPMI 1640 was added and samples were incubated at 37 °C for 48 h for biofilm formation [22]. At that moment, the biofilms were washed twice with PBS, and the following tests were performed: quantification of CFU/mL, XTT assay and total biomass quantification by crystal violet staining (CV staining).

The CFU/mL and XTT assay were performed as the same way as described previously. The CV staining was used to determine the total biomass of the biofilm formed [21]. For this, biofilms were washed twice with 200 μ L of PBS, fixed with 200 μ L of methanol for 15 min. Thereafter, the methanol was removed and the plates were maintained at 37 °C for approximately 20 min to dry. Then 200 μ L of 1% crystal violet dye were added and maintained for 5 min. The biofilms were washed with ultrapure water and, then, 33% acetic acid was added to remove the dye. A volume of 100 μ L was transferred to another 96-well microtiter-plate and the colorimetric analysis was measured at 570 nm in spectrophotometer (Thermo Plate / TP Reader) [21].

2.5. Statistical analysis

Data of CFU/mL of *C. albicans* were converted into base-10 logarithms. Since the data met the assumptions of normality and homoscedasticity, they were submitted to Two-way ANOVA with the treatments (groups of treatment) evaluated at different periods (24 h and 7 days after treatment) as main effects, followed by Tukey *post-hoc* ($\alpha = 5\%$).

Data related to the virulence factors of adhesion capacity and biofilm formation of *C. albicans* evaluated by XTT assay, CFU/mL, and CV staining also met the assumptions of normality and homoscedasticity. So, they were submitted to two-way ANOVA and Tukey *post-hoc* for multiple comparisons.

3. Results

3.1. Microbiological evaluation

According to the statistical analysis performed, there was no interaction between the factors treatment group and time interval, so the mean values of CFU/mL were pooled ($p > 0.05$) (Fig. 1). CIAIP-NE-mediated aPDT was as effective as the antifungal Nystatin in the reduction of the CFU/mL, which was equivalent to 1.4 and 2.0 \log_{10} , respectively, independent of the time interval assessed ($p = 0.136$ after 24 h and $p = 0.64$ after 7 days). Both treatments showed significant reduction in the cell viability in comparison with P-L- group ($p < 0.0001$).

3.2. Adhesion and biofilm formation of *C. albicans* after treatments

With regard to the adhesion capacity, the statistical analysis showed significant reduction of the CFU/mL for the groups treated with aPDT mediated by CIAIP-NE (P + L+) and NYS, which were equivalent to 1.0 and 2.3 \log_{10} , respectively, being different from each other and

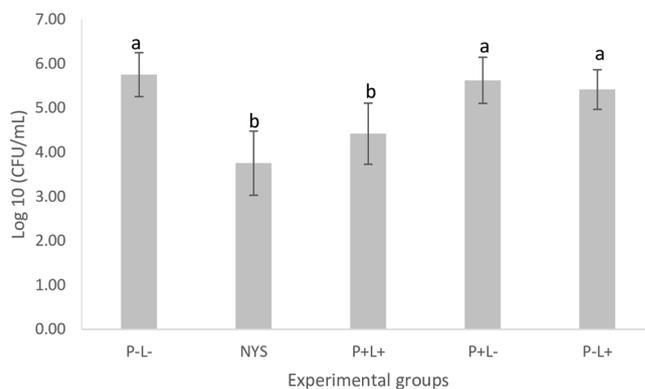


Fig. 1. Mean values and standard deviation of \log_{10} (CFU/mL) obtained for the control and experimental groups. Different superscript lowercase letters denote statistical difference between the experimental groups ($p < 0.05$). It was performed the two-way ANOVA followed by Tukey's test to compare the groups according to the different time intervals assessed. As there was not interaction between the factors treatment group and time interval, the mean values of CFU/mL were pooled ($p > 0.05$).

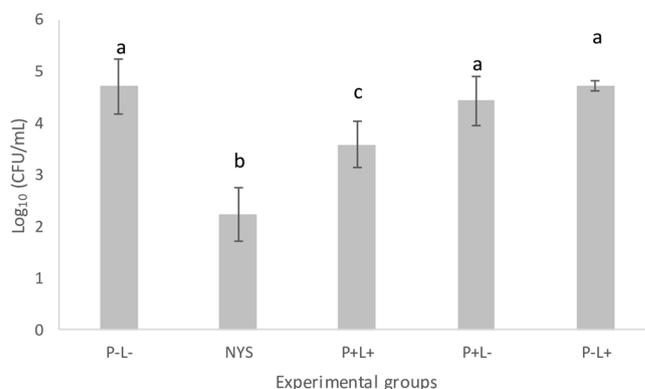


Fig. 2. Mean values and standard deviation of \log_{10} (CFU / mL) of *C. albicans* after the adhesion phase. Equal letters show statistical similarity between groups ($p > 0.05$). As there was not interaction between the experimental groups in relation to the time interval assessed, the data were pooled in the same bars. The data was submitted to two-way ANOVA followed by Tukey *post hoc*.

different from the P-L- ($p = 0.001$ and $p \leq 0.0001$, respectively). Additionally, the use of the CIAIP-NE or light alone was statistically similar to the control group ($p \geq 0.05$) (Fig. 2). The results obtained for the group of animals sacrificed 7 days after the end of the treatments were similar to those of 24 h.

Twenty-four hours after treatments, the groups submitted to aPDT and NYS showed a significant reduction in the cellular metabolism when compared to the P-L-, which were equivalent to 42% and 46% ($p \leq 0.0001$ and $p \leq 0.0001$), respectively. The groups submitted only to the application of light (P-L+) or PS (P + L-) were similar to the P-L-group ($p \geq 0.05$). Seven days after treatments, the reduction caused in the cellular metabolism by aPDT and NYS was statistically similar to each other, and were similar to the control group ($p \geq 0.05$) (Fig. 3).

For the biofilm formation, there was a significant reduction in the cell viability of the groups submitted to aPDT and NYS, which were approximately 1.2 \log_{10} and 3.5 \log_{10} , respectively, when compared with the control group ($p \leq 0.0001$ and $p \leq 0.0001$, respectively) in both period of time assessed. The other groups were similar to the control group (P-L-) (Fig. 4).

The analysis of cellular metabolism revealed that the cells from P + L+ (aPDT) and NYS groups presented values statistically similar to each other, and were statistically different from the control group ($p \leq 0.0001$ and $p \leq 0.0001$, respectively) (Fig. 5). The reductions of

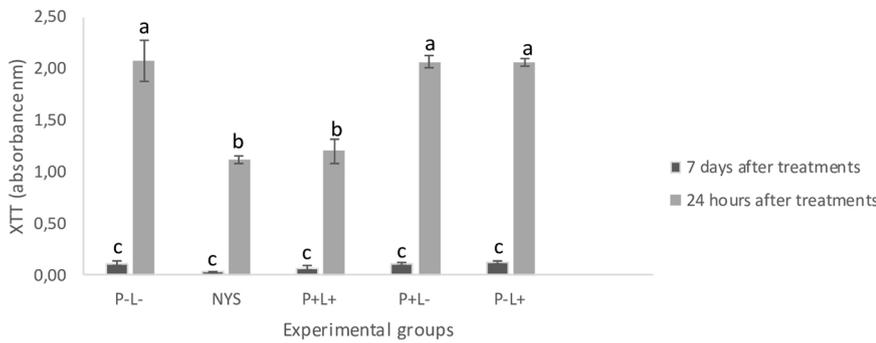


Fig. 3. Mean values and standard deviation of absorbance obtained by XTT assay (nm). Equal letters show statistical similarity between groups in the specific time interval assessed ($p > 0.05$). The values were submitted to two-way ANOVA. As there was interaction between the experimental groups in relation to the time interval assessed, the data were also submitted to Tukey post hoc.

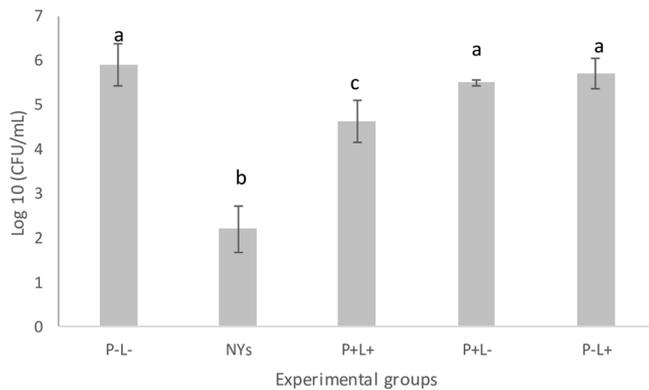


Fig. 4. Mean values and standard deviation of \log_{10} (CFU/mL) of *C. albicans* after biofilm formation. Equal letters show statistical similarity between groups ($p > 0.05$). As there was not interaction between the experimental groups in relation to the time interval assessed, the data were pooled in the same bars. The data was submitted to two-way ANOVA followed by Tukey post hoc.

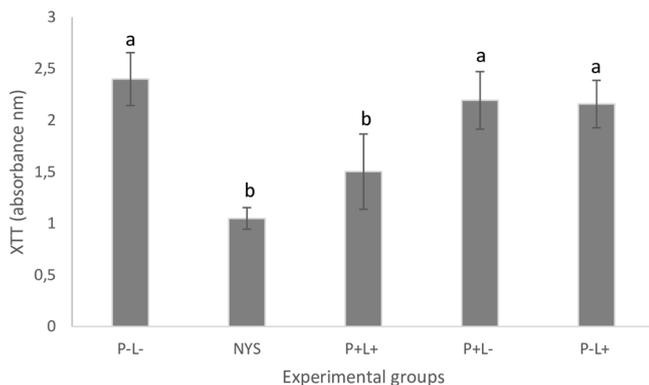


Fig. 5. Mean values and standard deviation of absorbance obtained by the XTT assay (492 nm). Equal letters show statistical similarity between groups ($p > 0.05$). As there was no interaction between the experimental groups in relation to the time interval assessed, the data were pooled in the same bars. The data were submitted to two-way ANOVA followed by Tukey post hoc.

the cell metabolism were equivalent to 45% and 62% for P + L+ and NYS groups, respectively. The other groups were similar to the control group (P-L-) (Fig. 5). These results were the same for both time intervals assessed.

As can be seen in the Fig. 6, the cells belonged to the groups of animals that received P + L+ and NYS treatments showed a significant reduction in the total biomass of biofilms, which were equivalent to 67% and 79%, when compared to the control group ($p = 0.002$ and $p = 0.0001$, respectively). The other groups were similar to the control group. These results were the same for both time intervals assessed.

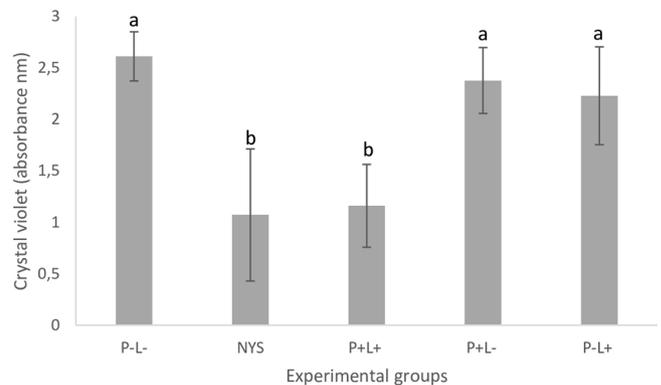


Fig. 6. Mean values and standard deviation of the total biomass assay (absorbance of CV staining at 570 nm) of *C. albicans* biofilm. Equal letters show statistical similarity between groups ($p > 0.05$). As there was no interaction between the experimental groups in relation to the time interval assessed, the data were pooled in the same bars. The data was submitted to two-way ANOVA followed by Tukey post hoc.

4. Discussion

In the present investigation, -CIAIP-NE-mediated aPDT was as effective as NYS in the treatment of oral candidosis in mice. The use of 31.7 μM of CIAIP-NE associated with a light dose of 100 J/cm^2 reduced 1.4 \log_{10} of CFU/ml, while NYS promoted 2.0 \log_{10} of reduction, for both time intervals evaluated. Carmello et al. [18], using the same animal model, reported that 5 consecutive applications of aPDT mediated by Photodithazine (PDZ) 100 mg/L with 37.5 J/cm^2 of light was able to reduce 3 \log_{10} of CFU/ml, while NYS promoted 3.2 \log_{10} . Furthermore, in a clinical trial [24], it was also verified that aPDT mediated by Photogem was as effective as Nystatin in the treatment of patients with denture stomatitis. These results demonstrated that aPDT is a promising therapy independent of the PS used.

The adhesion is an important virulence factor of *C. albicans* which allows the adherence of microorganisms in the host tissues and it is also a precursor stage to form a biofilm community and the extracellular matrix, starting the infection [11]. The most important *C. albicans* adhesion proteins are encoded by the ALS1 (Agglutinin like sequence), ALS3, HWP1 (Hypha Wall Protein) and EAP1 (enhanced adherence to polystyrene) genes [25]. In addition to adhesion, the proteins involved with the filamentation and quorum sensing of *C. albicans* also might have an important role in biofilm formation. Many of these proteins are encoded by genes regulated by transcription factors that are activated during biofilm formation, such as BCR1 (biofilm and cell wall regulator 1) and ZAP 1 (zinc-responsive transcription factor 1) and also during the dispersion of biofilm cells, such as Ume6, Pes1 and Nrg1 [25]. Therefore, a therapy that interferes in some of the phases involved in the adhesion and biofilm formation can be considered promising. The results of the present study reported that twenty-four hours after treatments, aPDT and NYS treatment were able to reduce 1.0 and 2.3

\log_{10} CFU/mL, respectively, of the cell viability of *C. albicans* during the adhesion in both period of time evaluates, being both treatments statistically similar. The XTT assay demonstrated that aPDT and NYS reduced 42% and 46%, respectively, of the cellular metabolism of *C. albicans* 24 h after the treatments. Regarding 7 days of the treatments, the groups treated with aPDT and NYS were able to maintain the reduction in the metabolic activity, which was equivalent to 38% and 46%, respectively. Likewise, Soares et al. [26] verified 5.20 \log_{10} of reduction in the cell viability of *C. albicans* with correspond a reduction in the adhesion of 61.5% to the bucco-epithelial cells (BECs) after Toluidine blue-mediated aPDT in vitro [26]. On the other hand, Alves et al. [21] did not find reduction in the adhesion capacity with respect to the cell viability, and the metabolic activity reduced just 15.8% for *C. albicans* submitted to one application of PDZ-mediated aPDT in vitro. The divergences in the results can be attributed to the different properties of each PS used and also the different protocols evaluated. According to our knowledge, the present investigation is the first in vivo study which verified that the aPDT is a possible alternative to inhibit *C. albicans* adhesion to abiotic surfaces.

It was observed that the treatments performed showed a significant reduction in the capacity of biofilm formation of *C. albicans*. Reductions in the cell viability, equivalent to 1.2 and 3.5 \log_{10} were observed for aPDT and NYS, respectively. The analysis of the cellular metabolism revealed similar reductions of the groups submitted to aPDT and NYS, which were equivalent to 45 and 62%, respectively. According to Alves et al. [21], the strains of *C. albicans* underwent to PDZ-mediated aPDT in vitro showed a reduction equivalent to 16% in the capacity of biofilm formation. In another in vitro study the aPDT mediated by toluidine blue was able to reduce 62% of the capacity of biofilm formation [27]. These findings and those observed here highlight the effectiveness of aPDT in reducing the capacity of biofilm formation of *C. albicans*, independent of the PS and the protocol used.

With respect to the total biomass of biofilms, aPDT and NYS treatment in vivo were able to reduce 67% and 79% of the total biomass, respectively. On the other hand, Alves et al. [21] did not observe reduction in the total biomass of the biofilms of *C. albicans* strains tested after aPDT in vitro. This data may be justified by the fact that Alves et al. [21] made only one application of aPDT while in the present study 5 consecutive session of aPDT was made. Other investigations reported the effectiveness of aPDT mediated by curcumin in reducing the total biomass of mono-species biofilms from clinical isolates of *Candida* spp. [28], and the total biomass of mixed-species biofilms formed on the acrylic resin samples [29]. The biofilm is a microbial community surrounded by a self-produced extracellular matrix with the capacity to adhere to biotic or abiotic surfaces. The microorganisms inside this community have some advantages such as, altered gene expression, which allows important phenotype characteristics to survive on hostile environments [30]. In addition, the extracellular matrix per se is responsible to protect the fungal cells inside the biofilms and it is the main constituent of the biomass of the biofilms [31]. As a consequence, the biofilms are resistant to conventional treatments. Therefore, the reduction of total biomass of biofilms observed in our study suggest that aPDT seems to have a good capacity for disarticulation of the biofilm structure, which could be a promising alternative approach to inactivate *C. albicans* biofilm.

The NYS treatment was also undertaken in the present work for comparative purposes, as it is a very common prescribed topical medication to treat oral candidiasis in agreement with The Infectious Diseases Society of America (IDSA) guidelines [5]. Topical medications, for instance NYS, are commonly prescribed in the first occurrences of OC and they are effective to relieve the clinical symptoms of the infection [32]. However, this drug shows temporary results, since the microorganism is not completely eliminated, probably due to the reduction of the drug concentration in the infected tissues, associated with the dilution effects of saliva and tongue movements, so the drug concentration is likely to be sub-therapeutic [34]. As a consequence, the

re-colonization of strains is frequent after treatment, which leads to the recurrence of infection, and multiple doses are necessary to treat the disease [33]. aPDT seems to have relevant advantages over conventional treatments of oral candidiasis due to the possibility of inactivation of resistant strains to conventional treatments, since this therapy involves the formation of non-specific ROS [34]. In the clinical field, the aPDT treatment can be performed and controlled by the professional, who can reinforce the importance of the oral hygiene. Therefore, the results of mentioned studies and those obtained in the present investigation reinforce that aPDT is a promising treatment of oral candidosis.

The effectiveness of CIAIP-NE in a murine model of oral candidosis was previously reported [17]. In the referred investigation it was used the same parameter of irradiation and PS concentration employed here, and it was obtained 2.26 \log_{10} of reduction after one session of aPDT [17]. This result does not corroborate with those found in the present study, since five consecutive sessions of aPDT promoted 1.4 \log_{10} of reduction. Likewise, another investigation [18] showed that 5 sessions of PDZ-mediated aPDT was less effective than only one application of PDZ-mediated aPDT [35]. It was expected that 5 sessions of aPDT, independent of the PS used, would culminate in a higher reduction of *Candida* viability, in comparison with a single application. It was not observed in the present investigation neither in the study performed by Carmello et al. [18]. Therefore, *Candida* cells seem to have the capacity of adaptation under oxidative stress by decreasing the cell membrane permeability of ROS or increasing the regulation of associated enzymes [36].

Considering the findings of the present investigation, CIAIP-NE seems to be an

effective PS to use in aPDT, since low concentrations of the PS was used and 5 successive applications were as effective as the antifungal NYS to treat mice with experimental OC. With regard to the virulence factors, our results suggest that aPDT in vivo was able to reduce de capacity of biofilm formation on abiotic surface decreasing the cell viability, cellular metabolism and the total biomass of the biofilm. Taken together, the results reinforce the idea that aPDT may be a good option to inactivate *C. albicans*, impairing the dissemination of infection to other sites of the host body.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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