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Assessment of sensitivity of selected *Candida* strains on antimicrobial photodynamic therapy using diode laser 635 nm and toluidine blue – *In vitro* research

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ABSTRACT

Background: Photodynamic therapy is believed to be a promising treatment for *Candida* infections. This study evaluated the efficacy of antimicrobial photodynamic therapy (aPDT) using the 635 nm diode laser light and toluidine blue (TB) in the elimination of selected *Candida* species cultured on acrylic surface.
Methods: 108 acrylic plates (Methyl Methacrylate Polymer, routinely used for the production of prosthetic dentures) were placed in three sterile Petri dishes and poured with prepared suspensions of *Candida* strains: *C. albicans*, *C. glabrata*, and *C. krusei*. After all procedures of fungi incubation, fungal biofilm was visible on the plates' surfaces. The acrylic plates were divided into nine study groups (B) and nine control groups (K) for further experiments. In the study groups, the acrylic plates with fungal biofilm were immersed in TB and afterwards laser irradiation was applicated with different exposure parameters (groups: B1 – 400 mW, 24 J/cm^2, 30 s; B2 – 300 mW, 18 J/cm^2, 30 s; B3 – 200 mW, 12 J/cm^2, 30 s) separately for each *Candida* species. The control groups contained following parameters: no exposure to laser light or TB, treatment only with TB without laser irradiation, or only laser irradiation without previous immersion in TB. Calculations of colony forming units (CFUs) were conducted by using aCOlyte (Synbiosis). Differences in CFUs were analyzed by the Wilcoxon test.
Results: In all study groups, the reduction in CFUs was statistically significant. The differences in CFUs before and after intervention were insignificant. The K3 $_{C.a.}$ control group showed a statistical reduction of *Candida albicans* after laser irradiation.
Conclusion: Our study confirmed the efficacy of aPDT against *C. albicans, C. glabrata* and *C. krusei* being dependent on the laser parameters and the type of fungus. The advantage of this study is the validation of aPDT effectiveness in *in vitro* studies to transpose this data into future clinical trials using photodynamic therapy in the treatment of oral candidiasis.

1. Introduction

Candidiasis is one of the most common diseases of oral mucosa. Leading etiologic factors of oral fungal infections are yeast-like fungi of the genus *Candida*: mainly *Candida albicans* (50–70% of cases), less frequently *C. glabrata, C. tropicalis, C. parapsilosis*, or *C. krusei* [1–4]. In healthy individuals, *C. albicans* exists in oral cavity as a harmless commensal without causing disease, however, in some situations (especially in immunosuppressed patients), it can become virulent and cause candidiasis. There are several systemic and local risk factors for the evolvement of oral candidiasis (OC), such as immunosuppressant or chronic broad-spectrum antibiotic therapies, diabetes mellitus, pregnancy, premature very low birth weight infants, immunocompromised individuals, HIV infections, long-term catheterization, invasive medical procedures, kidney affections, chronic local steroid treatments, xerostomy, smoking, high carbohydrate diet, denture wearing (about

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60–65% of the patients using dentures suffer from stomatitis associated with *Candida* infection, which remains the most frequent form of OC), poor oral hygiene, etc. [1–5]. Moreover, in recent years, an increase in the incidence of OC is observed, which is associated primarily with demographic changes as well as the development of medicine and the pharmaceutical industry. Severe, long-term treatments in addition to the population aging, the introduction of invasive and aggressive anticancer therapies, and the development of transplantology favors the development of both superficial and systemic mycoses [4,6]. This growing problem of OC development forces clinicians to better understand *Candida* spp. virulence and antifungal treatment [1].

Successful treatment of candidiasis is possible with careful and individual analysis of each clinical case for determination of the primary or secondary cause of this infection.. Firstly, local and/or general predisposing factors for the development of mycosis should be eliminated or controlled for, if possible.. Then, it is also necessary to carry out an appropriate antifungal treatment (generally including a mycological test with an antimycogram) [2–4,6]. The period of pharmacotherapy should last a minimum of 2-3 weeks because in the case of too short a treatment or the use of inappropriate doses, patients may develop recurrent candidiasis and the emergence of drug resistance to yeasts. Also, it is important that the treatment covers the oral mucosa and denture plate, if a patient uses one [4,6]. Currently, medicine does not have an ideal antimycotic which would be able to withstand all virulence factors of different fungi. Increasing resistance among *Candida* spp. to available antifungal drugs is a serious challenge for modern medicine. Due to this unfavorable phenomenon, there is a need to develop new methods of treatment of OC [1]. A promising alternative to pharmacological antifungal therapy is antimicrobial photodynamic therapy (aPDT) with the use of photosensitizers and appropriated length of light waves, e.g. laser [7]. Antimicrobial photodynamic therapy is based on the interaction between three components: a photosensitizer (e.g. toluidine blue), light at a wavelength corresponding to its maximum absorption, and molecular oxygen. The stimulated photosensitizer initiates a cascade of processes that result in the reactive oxygen species responsible for the destruction of pathogenic microorganisms. The advantages of aPDT include: no development of microbial resistance (possibility of repeating the therapy) and their rapid elimination, ease of use, and safety (no cytotoxic effects on host tissues) [7–10].

The aim of this study was to evaluate the efficiency of antimicrobial photodynamic therapy using the 635 nm diode laser and toluidine blue as a photosensitizer against selected *Candida* species cultured on acrylic plates.

2. Material and methods

The research was conducted in the Department of Microbiology and Virology in Sosnowiec (Medical University of Silesia, Poland) and the Department of Periodontal Disease and Oral Mucosa in Zabrze (Medical University of Silesia, Poland).

2.1. Candida strains

In this *in vitro* experimental study, three reference strains of *Candida* spp. were used: *C. albicans* ATCC 10231 (American Type Culture Collection), *C. glabrata* ATCC 15126, and *C. krusei* ATCC 14243. Prepared suspensions were cultured in the liquid Sabouraud dextrose agar, with a density of 0.5 on the McFarland scale. The density was measured with the densitometer (Densi-La Meter II, Erba Lachema, Czechia). Prepared suspensions, each 30 ml, were placed in sterile, calibrated, and conical test tubes.

2.2. Acrylic plates

The experimental 108 acrylic plates were prepared by using thermally activated Methyl Methacrylate Polymer (acrylic resin) routinely used for the production of prosthetic dentures, with the dimension of 10 × 10 x 1 mm each. The way they were made, the composition and the porosity, corresponded to the mucosal part of the traditional acrylic dentures. Before they were used in the study, they had been sterilized in an autoclave at 134 °C for 40 min and pressure value 2.1 atm.

Fig. 1. The acrylic plates soaked in suspensions of Candida strains.

2.3. Biofilm formation

The acrylic plates were placed in six sterile Petri dishes (18 plates in each Petri dish) and poured with suspensions of *Candida* strains (Fig. 1). The acrylic plates were arranged, so that they did not lie on top of each other. The samples were immerged in *Candida* suspensions for 3 h in 21 °C. After that, they were relocated from the suspensions into sterile metal trays and further incubated for 72 h in 37 °C in order to amplify the *Candida* strains and the biofilm formation. After the procedures, fungal biofilm was visible on the plates' surfaces.

2.4. Study and control groups

The acrylic plates were divided into study groups (B) and control groups (K) for further experiments. The Petri dishes containing the Sabouraud agar medium with added 4% glucose (BTL, Łódź, Poland) were divided with a marker (on glass) into two equal parts and were referred to as field 1 and 2. In each study and control group, before any intervention, three acrylic plates with *Candida* biofilm were attached for 10 s to the surface of the field 1. In nine study groups, laser irradiation was applicated with different parameters of light separately for each *Candida* species (*C. albicans* – C.a., *C. glabrata* – C.g., *C. krusei* – C.k.). In groups B1, B2, and B3, the plates were immersed in TB (Gel Universal, PACT) (Fig. 2) for 60 s and the photosensitizer was activated for 30 s with a different output power. A diode laser SMART^{m} PRO (Lasotronix, Piaseczno, Poland) emitting a continuous wave (CW) at a wavelength of 635 nm was used as a light source. The irradiation was performed with 8 mm in diameter glass optical fiber head at a distance of 1 mm (without contact). The study groups were as follows:

- Study group B1 $_{C.a., C.g., C.k.}$ - TB applied to a biofilm presented on the acrylic plates, which was sequentially irradiated with 635 nm light, CW, **400 mW** power [P], for 30 s [t] (energy density 24 J/cm^2 [E]) (Fig. 3);
- Study group B2 $_{C.a., C.g., C.k.}$- TB applied to a biofilm presented on the acrylic plates, which was sequentially irradiated with 635 nm light, CW, **300 mW** power, for 30 s (energy density 18 J/cm^2);
- Study group B3 $_{C.a., C.g., C.k.}$ - TB applied to a biofilm presented on the acrylic plates, which was sequentially irradiated with 635 nm light, CW, **200 mW** power, for 30 s (energy density 12 J/cm^2).

Activated photosensitizer was left for next 30 s, then washed in

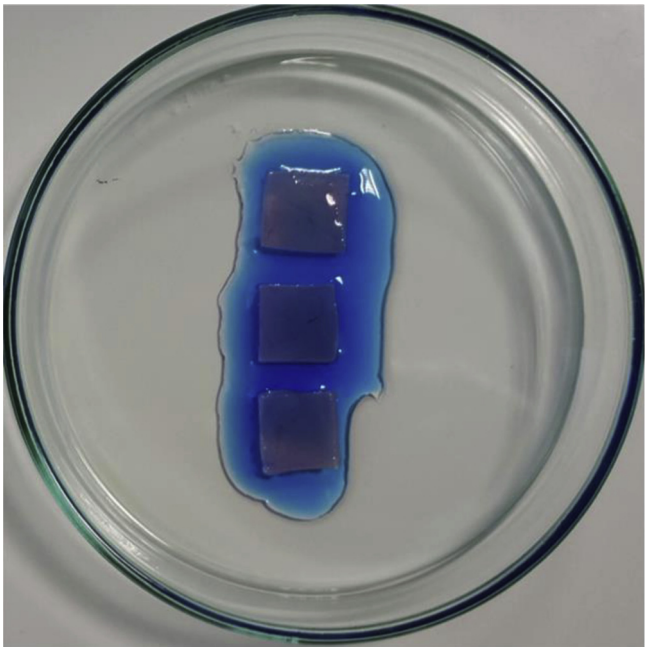

Fig. 2. Acrylic plates immersed in TB (Gel Universal, PACT).

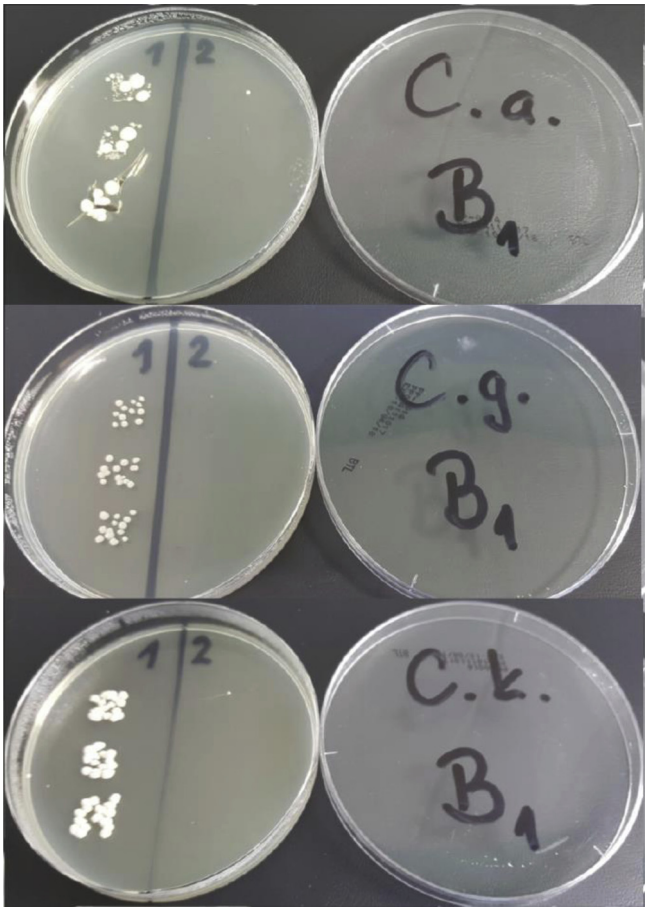

Fig. 3. B1 C.a., C.g., C.k. – before (1) and after TB was applied to a biofilm presented on acrylic plates, which was sequentially irradiated with 635 nm light, CW, 400 mW power, for 30 s (energy density 24 J/cm^2) (2).

0.9% NaCl solution for 10 s, dried and then attached again for 10 s to the same Petri dish, but to the field 2 this time. Control groups were created as follows:

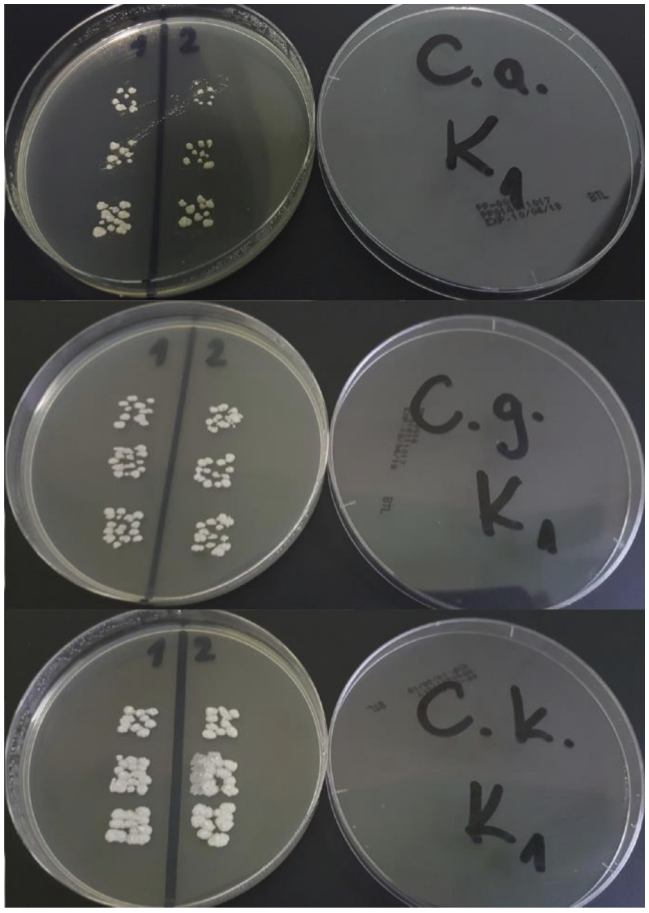

Fig. 4. K1 C.a., C.g., C.k. – without any intervention (no exposure to laser light or photosensitizer), first touch on field 1 and second touch on field 2.

- Control group K1 $_{C.a., C.g., C.k.}$ – without any intervention (no exposure to the laser light or photosensitizer), first touch on the field 1 and second touch on the field 2 (Fig. 4);
- Control group K2 $_{C.a., C.g., C.k.}$ – acrylic plates with fungal biofilm were treated only with the photosensitizer for 1 min without laser irradiation;
- Control group K3 $_{C.a., C.g., C.k.}$ – only laser irradiation (635 nm, CW, 400 mW power, 30 s, 24 J/cm^2 energy density) without previous immersion in the photosensitizer.

Then, all Petri dishes were incubated in 37 °C for 72 h. Finally, after the removal from the incubator, photographic documentation was prepared and the calculations of colony forming units (CFUs) were conducted using aCOlyte (Synbiosis).

Differences in CFUs were analyzed by the Wilcoxon test. Differences between a number of colonies of *Candida albicans, glabrata,* and *krusei* after the photodynamic therapy in the study groups were analyzed by the Mann-Whitney U test. A P value of ≤ 0.05 was considered to indicate a statistically significant difference.

3. Results

The results of the reduction in *Candida* selected species for the study and control groups are shown in Tables 1 and 2. In all study groups, the reduction in CFUs was statistically significant (Table 1). In almost all control groups, the differences in CFUs before and after intervention were insignificant. One control group – K3 $_{C.a.}$ – showed a statistical reduction of *Candida albicans* after laser irradiation (400 mW power, 30 s, 24 J/cm^2 energy density) (Table 2). The differences between a

Table 1
Results of reduction in *Candida* selected species for the study groups. Statistically significant difference (Wilcoxon test, $p \leq 0.05$).

Parameters of light for photodynamic therapy	635 nm, CW, t = 30 s, P = 400 mW, E = 24 J/cm^2						635 nm, CW, t = 30 s, P = 300 mW, E = 18 J/cm^2						635 nm, CW, t = 30 s, P = 200 mW, E = 12 J/cm^2					
Study groups	B1 C.a.		B1 C.g.		B1 C.k.		B2 C.a.		B2 C.g.		B2 C.k.		B3 C.a.		B3 C.g.		B3 C.k.	
Before and after irradiation	before	after	before	after	before	after	before	after	before	after	before	after	before	after	before	after	before	after
Average number of colonies CFU for 6 trials (n = 6)	24.00	0.50	13.67	0.33	13.50	0.50	40.33	3.00	20.50	0.67	11.67	0.50	10.50	6.67	11.67	5.67	10.17	6.33
Standard Deviation	15.36	0.55	1.86	0.52	2.59	0.55	16.86	2.68	4.93	0.52	5.16	0.83	2.59	1.03	1.63	1.37	0.75	1.36
Median	16.00	0.50	14.00	0.00	14.00	0.50	39.50	2.00	19.50	1.00	10.00	0.00	11.00	7.00	12.00	5.50	10.00	6.50
Maximum	45.00	1.00	16.00	1.00	17.00	1.00	66.00	8.00	26.00	1.00	22.00	2.00	14.00	8.00	14.00	8.00	11.00	8.00
Minimum	11.00	0.00	11.00	0.00	10.00	0.00	22.00	1.00	13.00	0.00	8.00	0.00	7.00	5.00	9.00	4.00	9.00	4.00
P-value	≤ 0.05		≤ 0.05		≤ 0.05		≤ 0.05		≤ 0.05		≤ 0.05		≤ 0.05		≤ 0.05		≤ 0.05	

Table 2
Results of reduction in *Candida* selected species for the control groups. Statistically significant difference only in K3 C.a. group (Wilcoxon test, $p \leq 0.05$).

Group parameters	No exposure to laser light or photosensitizer						Only with photosensitizer for 1 minute						Only laser irradiation (635 nm, CW, 400 mW power, 30 s, 24 J/cm^2 energy density)					
Control groups	K1 C.a.		K1 C.g.		K1 C.k.		K2 C.a.		K2 C.g.		K2 C.k.		K3 C.a.		K3 C.g.		K3 C.k.	
Before and after the action	before	after	before	after	before	after	before	after	before	after	before	after	before	after	before	after	before	after
Average number of colonies CFU for 6 trials (n = 6)	21.17	20.83	13.83	13.50	13.17	12.67	35.00	34.83	14.17	14.83	6.67	6.00	10.50	8.67	11.50	10.67	10.00	9.67
Standard Deviation	13.44	13.29	2.40	2.17	2.7	2.42	15.67	15.10	15.11	4.26	2.94	3.74	2.59	2.07	1.64	1.03	0.63	1.36
Median	17.00	17.00	14.50	14.00	14.00	13.00	36.50	36.00	12.00	13.50	7.00	7.00	11.00	8.00	11.50	11.00	10.00	9.50
Maximum	41.00	40.00	16.00	16.00	17.00	16.00	52.00	51.00	22.00	21.00	10.00	10.00	14.00	12.00	14.00	12.00	11.00	12.00
Minimum	7.00	7.00	10.00	10.00	10.00	10.00	18.00	18.00	10.00	10.00	2.00	1.00	7.00	7.00	9.00	9.00	9.00	8.00
P-value	0.18		0.18		0.18		0.72		0.29		0.18		≤ 0.05		0.27		0.50	

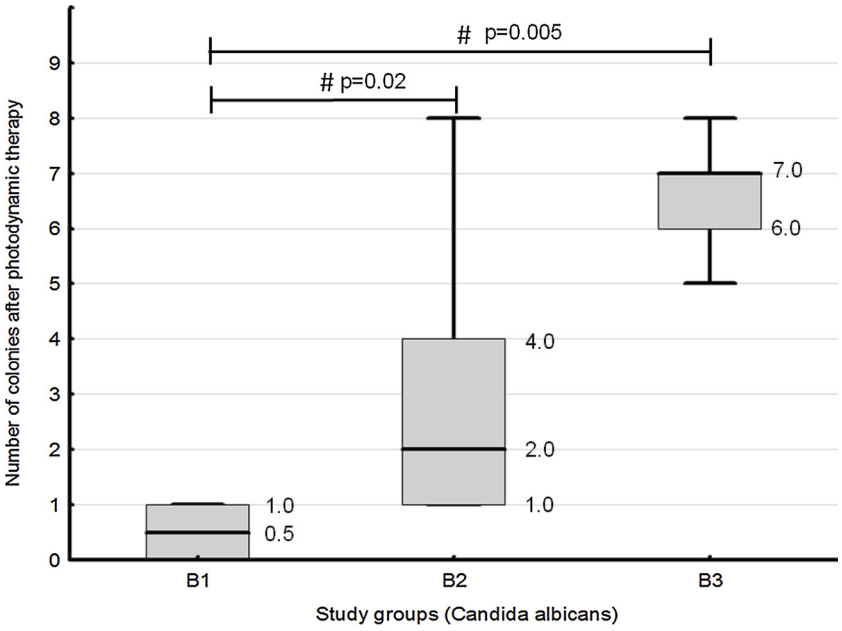

Fig. 5. Differences between number of colonies of *Candida albicans* after the photodynamic therapy in the study groups (group B1 – 400 mW, 24 J/cm^2, 30 s, group B2 – 300 mW, 18 J/cm^2, 30 s, group B3 – 200 mW, 12 J/cm^2, 30 s,). # $P < 0.05$; Mann-Whitney U test. Medians are shown as lines, 25th and 75th percentiles are boxes, the whiskers represents minimum and maximum.

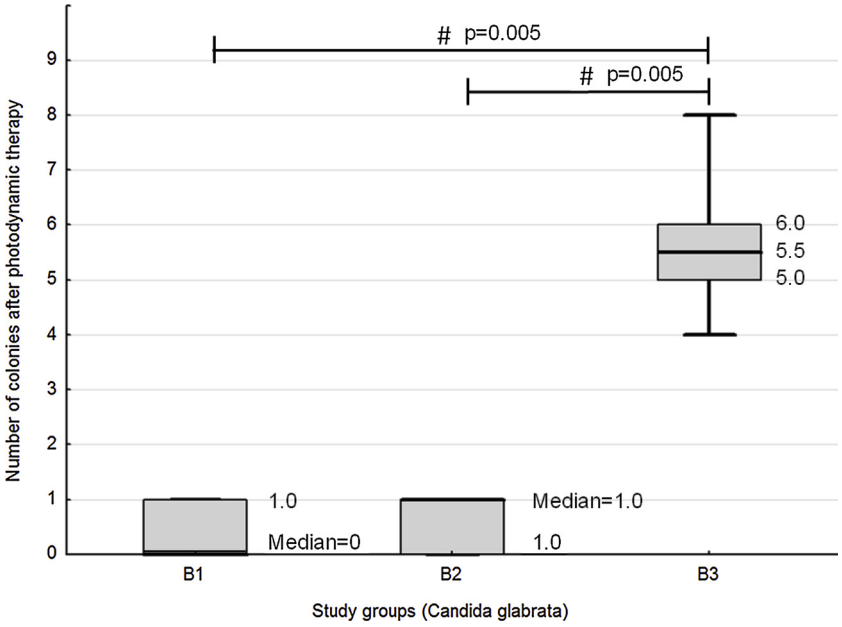

Fig. 6. Differences between number of colonies of *Candida glabrata* after the photodynamic therapy in the study groups (group B1 – 400 mW, 24 J/cm^2, 30 s, group B2 – 300 mW, 18 J/cm^2, 30 s, group B3 – 200 mW, 12 J/cm^2, 30 s,). # $P < 0.05$; Mann-Whitney U test. Medians are shown as lines, 25th and 75th percentiles are boxes, the whiskers represents minimum and maximum.

number of colonies of *Candida albicans, glabrata*, and *krusei* after the photodynamic therapy in the study groups are shown in Figs Fig. 55, Fig. 66 and Fig. 77 .

4. Discussion

Oral candidiasis, currently regarded as a civilization disease, is mainly associated with the host's lack of immunological response. Because of immunosuppression (systemic diseases, long-term pharmacological treatment) and local factors (mainly the use of acrylic removable dentures), the yeast strains identified in the *Candida* infection are increasingly non-*albicans*: *C. glabrata, C. krusei, C. dubliniensis,* or *C. tropicalis.* The occurrence of these strains results in higher virulence of pathogens and often in natural resistance to many antifungal drugs. Frequent multiresistance, also acquired, and numerous side effects of drugs used in the treatment of candidiasis create the need to search for new methods of eliminating yeast [1–6].

Many previous studies recommended aPDT as a promising alternative to pharmacological antifungal therapy with positive results [11–18]. Due to the specific structure of the fungal cell (the size of the microorganism, the presence of the nucleus and the cell wall), yeast is less sensitive to the effects of photodynamic therapy. Therefore, only some photosensitizers are used, mainly: methylene blue (MB), TB, indocyanine green (ICG), malachite green (MG), or Photogem®, and strictly selected light sources [12,16].

The best-known photosensitizer in relation with *C. albicans* is MB. It is used most often in *in vitro* studies with planktonic solutions of the microorganisms suspended in liquid Sabouraud's substrates. As an example, in the study by Ferreira et al. (2016), MB and the 660 nm diode laser with a power of 690 mW and fluence of 30 J/cm^2, 60 J/cm^2 and 120 J/cm^2 were used. It showed complete elimination of fungi with the fluence value of 60 J/cm^2 [11].

Similar results were obtained in the study by Kato et al. (2013) where MB and the 660 nm laser with an output power of 30 mW were

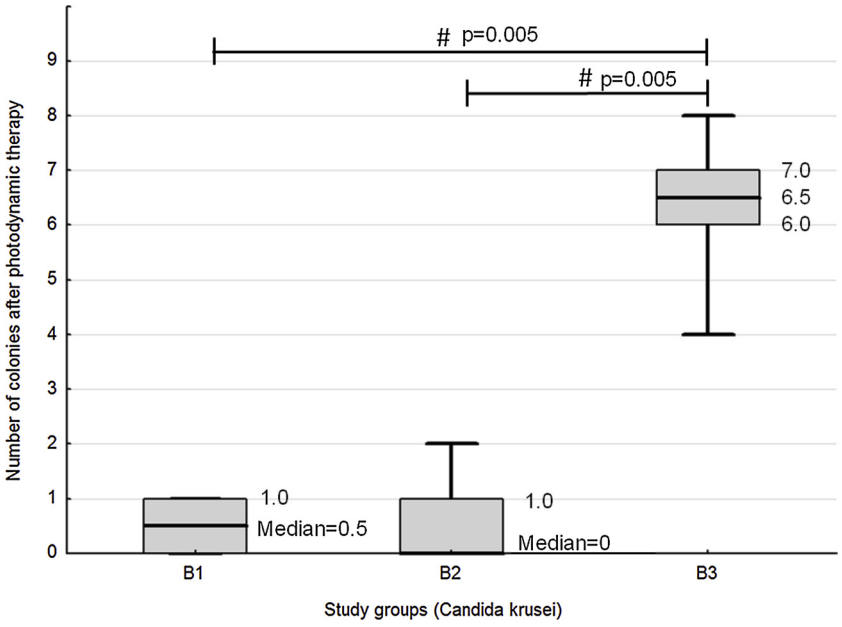

Fig. 7. Differences between number of colonies of *Candida krusei* after the photodynamic therapy in the study groups (group B1 – 400 mW, 24 J/cm^2, 30 s, group B2 – 300 mW, 18 J/cm^2, 30 s, group B3 – 200 mW, 12 J/cm^2, 30 s,). # $P < 0.05$; Mann-Whitney U test. Medians are shown as lines, 25th and 75th percentiles are boxes, the whiskers represents minimum and maximum.

used. The time of exposure after the addition of photosensitizer to the skin was 10 min. The tested energy densities were 9 J/cm^2, 18 J/cm^2 and 27 J/cm^2. The doses of 18 J/cm^2 and 27 J/cm^2 showed statistically significant reduction of the number of colonies of *C. albicans,* but they did not eliminate them completely [12].

Azizi et al. (2016) compared various combinations of MB and ICG with or without laser irradiation, with different exposure parameters, and with nystatin and chlorhexidine (CHX) against *C. albicans*. Results revealed that laser application (808 nm, 100 Hz pulse repetition rate) together with ICG caused the highest reduction in *C. albicans* CFUs. Second best result was achieved in the group treated with nystatin, and slightly weaker in MB group (660 nm, 100 Hz pulse repetition rate). In all groups, no total elimination of yeasts has been demonstrated. However, the study showed the possibility of using other photosensitizers than MB, such as the very effective ICG [13].

Souza et al. (2010) assessed the effectiveness of aPDT on the elimination of *C. albicans*. They worked with variable energy densities and TB, MB, and MG as photosensitizers. A 660 nm laser, 350 mW with energy densities of 15.8 J/cm^2, 26.3 J/cm^2 and 39.5 J/cm^2 was used. The best results were obtained in the group with TB and energy density of 39.5 J/cm^2, weaker with MB, and the smallest reduction occurred with MG. This study confirmed that TB, MB, and MG were effective photosensitizers in aPDT against *C. albicans* as well as that unalterable results depended on the laser energy, which was in line with our study [14].

In addition to the *C. albicans* strain, the sensitivity of other yeast (*C.dubliniensis, C.krusei, C.tropicalis.*) to aPDT with MB was also studied. In the research of Souza S.C. et al. (2006), a 685 nm laser, 350 mW, with the energy density of 28 J/cm^2 and a 5-minute timespan to start irradiation was used. Significant elimination of all tested strains has been demonstrated, which was also in line with our research [15].

There are very few *in vitro* studies assessing the effectiveness of aPDT in the elimination of yeast in the biofilm structure. One of the most interesting is the study of Sousa A.S. et al. (2016) assessing the elimination of *C. albicans* biofilm grown on acrylic plates. They used MB and a 660 nm laser (CW, fluency 34 J/cm^2 and 120 s of irradiation; 68 J/cm^2 and 240 s of irradiation; 137 J/cm^2 and 480 s of irradiation; 171 J/cm^2 and 600 s of irradiation), and also Proporphyrin IX and a 630 nm laser (same settings of the physical laser parameters). aPDT with MB in the group with the strongest dose of energy and the longest time of laser irradiation showed significant reduction of *C. albicans* from biofilm to acrylic, however, it did not eradicate them. Protoporirin IX did not show any efficacy in the biofilm structure [16].

On the basis of the above studies, our experiment was planned and carried out to assess the possibility of elimination of yeasts most frequently detected in prosthetic dentures, i.e. *C. albicans*, *C. glabrata*, and *C. krusei.* As a structure, a 3-day biofilm was selected on acrylic plates as a more similar situation to the real ones prevailing in the oral cavity. The results of the research are comparable to those obtained earlier by other authors. They showed complete elimination only in groups with the strongest physical laser parameters and only partial (depending on energy density) in other groups [8,14]. The obtained data confirms the huge effectiveness of TB in the described experiment, but it also confirms the observations of other authors, meaning the necessity of using high doses. Further detailed clinical studies are necessary to develop an appropriate aPDT algorithm for the daily treatment of oral mycosis, including the decontamination of prosthetic dentures as well as the oral mucosa of patients. Infected prosthetic dentures could be disinfected by a single-pass treatment with TB and appropriate laser parameters. It can be hypothesized that in the case of an infection of the mucous membrane, especially in the localized forms of candidiasis, using TB and then performing laser exposure, it could deactivate the fungi.

One of the few *in vivo* studies on mice by Freire et al. (2016) showed that photodynamic therapy using MB and new methylene blue (NMB) with diode laser at a wavelength of 660 nm and additionally combined with potassium iodide (KI) could be an effective method of the OC treatment. The applied light doses were 10 J, 20 J, 40 J and 60 J. The results showed the best yeast reduction in the group of MB + KI and the light dose of 40 J. Such yeast reduction was also visible in NBM without KJ group with the light dose of 60 J. These two groups were then used *in vivo* in a mouse model, showing almost full eradication of *Candida albicans*, especially in the group MB + KI + 40 J, after 5 days of treatment [8].

The studies of *in vivo* published to date have been carried out mainly on animal models (mice). Examples of such studies are the works of Freire et al. and Kato et al. in which the clinical efficacy of MB was obtained in combination with a 660 nm laser [8,12]. The doses of the used energy resulted from the data collected in the preceding *in vitro* tests (described above). aPDT was used once a day for 5 days [8]. In addition, increased sensitivity of yeast and strains to H_2O_2 and fluconazole, and reduced ability to create the so-called german tube forms associated with the initiation of the formation of a micelle form (more virulent) [12].

Clinical patient trials with prosthetic stomatitis were conducted by

Mima et al. (2012). They compared the efficacy of conventional Nystatin 100,000 IU with aPDT using Photogem® and LED 455 nm with the energy density of 122 J/cm^2 on the mucous membrane (4 times per day for 15 days), and 37.5 J/cm^2 on the plate of the infected prosthesis (3 times per week for 15 days) [17]. Both methods turned out to be equivalent.

In another clinical study by Scwingel et al. (2012), the efficacy of a single aPDT therapy with TB and a 660 nm laser (30 mW, 7.5 J/cm^2) was compared with systemic therapy having Fluconazole 0.1 g once per day for 14 days in HIV-patients. Both groups achieved efficacy against *Candida*, but only aPDT prevented relapse in monthly observations [18].

Most of the research on the antimicrobial modalities of aPDT used in dentistry are from *in vitro* studies; however, the meta-analysis of few *in vivo* studies proved that aPDT used as adjuvant to the chemo-mechanical therapy successfully abridged the microbial fill of an infected root canal system [19]. This data gives also high hopes for the possibility of using the results of this study in future clinical practice. Nevertheless, further randomized clinical trials targeted on the consistent aPDT parameters are desired.

It is also worth mentioning that new technologies, such as the use of nanoparticles, offer a novel and crucial approach for the treatment of microbial periodontal infections [20].

In the study of Sakima et al., encapsulated curcumin in polymeric nanoparticles was used as an alternative method for oral candidiasis in female mice, which revealed that aPDT application is a safe procedure for this condition [21].

The usual antifungal drugs used for oral candidiasis have shown disadvantages. Therefore, with the development of resistant strains, new therapies have been investigated. One of them is aPDT. It is presented as a new and promising antifungal therapy in many *in vitro* and in few *in vivo* studies; however, more research (especially *in vivo*) is necessary to develop this method.

5. Conclusion

The findings are as follows: the efficacy of aPDT against *C. albicans, C. glabrata,* and *C. krusei* has been confirmed; the highest antimycotic efficacy was obtained by using laser beam with the parameters of 400 mW, 24 J/cm2 and 30 s; toluidine blue with the appropriate laser parameters shows antifungal activity against the given strain; the outcome of the photodynamic therapy mostly depends on the laser parameters and the type of fungus.

The advantage of this study is the possibility of using the antimicrobial photodynamic therapy in a clinical environment in patients with Candidiasis of the oral mucosa. The data does not reflect all aspects that can be directly transposed to clinical trials, but nevertheless allows to presume what effects of a successful treatment can be expected in the trials. The results confirm the clinical usefulness of aPDT and demonstrate the utility of photodynamic therapy in patients with the inflammatory diseases of oral mucosa, including candidiasis. Future clinical studies are necessary to confirm our preclinical results.

Declaration of competing interest

The authors have no conflict of interest to declare.

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