Effect of different wavelengths and dyes on Candida albicans: In vivo study using Galleria mellonella as an experimental model

Effect of different wavelengths and dyes on *Candida albicans*: *In vivo* study using *Galleria mellonella* as an experimental model

Elisabetta Merigo[a, c, *]

elisabetta.merigo@gmail.com

Stefania Conti[b]

Tecla Ciociola[b]

Carlo Fornaini[a, c]

Luciano Polonelli[b]

Giuseppe Lagori[a, c]

Maddalena Manfredi[a]

Paolo Vescovi[a]

[a]Unit of Oral Pathology, Medicine and Laser Surgery, Department of Biomedical, Biotechnological and Translational Sciences (S.Bi.Bi.T.), University of Parma, Parma, Italy

[b]Unit of Microbiology and Virology, Department of Biomedical, Biotechnological and Translational Sciences (S.Bi.Bi.T.), University of Parma, Parma, Italy

[c]MicOralIS Laboratory EA7354 − UFR of Odontology, University of Nice-Sophia Antipolis, Nice, France

*Corresponding author at: Polo Clinico di Odontostomatologia − Unità di Odontostomatologia, Dipartimento di Scienze Biomediche, Biotecnologiche e Traslazionali (S.Bi.Bi.T), Via Gramsci, 14, 43126 Parma, Italy.

Abstract

Background

Studies on photodynamic inactivation against microorganisms had a great development in recent years. The aim of this work was to test the application of different laser wavelengths with or without different photosensitizing dyes on *Candida albicans* cells *in vitro* and in photodynamic therapy protocols *in vivo* in larvae of *Galleria mellonella*.

Methods

Laser application was realized on *C. albicans* cells suspended in saline solution or cultured on solid medium for the *in vitro* study, and in a model of *G. mellonella* candidal infection for the *in vivo* study. Three wavelengths (650, 405, and 532 nm) were used in continuous mode with different values of applied fluences: 10, 20 and 30 J/cm^2 for the *in vitro* study and 10 J/cm^2 for the *in vivo* study, without and with photosensitizing dyes.

Results

No growth inhibition was obtained on yeast cells in saline solution without photosensitizers. The maximum inhibition of growth (100%) was obtained with 405 nm diode laser and curcumin at any used fluence. No growth inhibition was observed for yeast cells cultured on solid medium after laser application without dyes. An inhibition was observed after laser application when curcumin and erythrosine were added to the medium.

The survival curves of *G. mellonella* larvae infected with *C. albicans* with or without the different dyes and after laser application showed a statistically significant difference ($p < 0.001$) in comparison with the proper control groups.

Conclusions

These results show the efficacy of photodynamic inactivation exploiting a suitable combination of light and dyes against *C. albicans* and the potential of photodynamic therapy for the treatment of candidal infections.

Keywords: *Candida albicans*; Diode Hlokaser; Photodynamic inactivation; Photodynamic therapy; Photosensitizers

1 Introduction

PhotoDynamic Therapy (PDT), discovered over 100 years ago by Oskar Raab, is a therapeutic cytotoxic technique applied for the treatment of infectious diseases and tumors since the '70 s [1,2]. The cytotoxic effect is achieved through local application or systemic administration (oral or intravenous) of photosensitizing agents followed by irradiation using visible light with emission spectrum appropriate to the absorption spectrum of the photosensitizer, in the presence of oxygen [3]. This will induce oxidation phenomena with irreversible selective destruction of proteins, lipids, nucleic acids and other cellular components.

Three basic elements are needed for the occurrence of a photodynamic reaction [4]: a photosensitizer localized in a target cell or tissue; a light source of specific wavelength, required to activate the photosensitizing molecule; molecular oxygen, which is essential for reactive oxygen species (ROS) generation.

Some organic molecules of biological origin can act as photosensitizers as they present a good quantum yield of triplet formation and a lifetime of this excited state relatively long (even hundreds of microseconds).

The requirements of an optimal photosensitizer are multiple: it should be non-toxic and show local toxicity only after light activation; moreover, it should present highly selective accumulation and high quantum yield of singlet oxygen production [5,6].

Most photosensitizers are activated by red light between 630 and 700 nm [7]. In the past, the activation was obtained by means of different light sources, such as argon laser, potassium titanyl-phosphate laser (KTP), or Nd:YAG laser; currently, it is more common to use diode laser, cheaper and easier to handle and transport, or light-emitting diodes (LED) [8–12].

The efficacy of photodynamic inactivation against microorganisms (Antimicrobial PhotoDynamic Therapy, APDT) has been widely reported in the last decades [13,14]. APDT showed also a promising potential in the treatment of fungal infections [15–17].

Recently, the invertebrate model *Galleria mellonella* has been used to study *in vivo* APDT against bacteria and fungi, alone or in combination with conventional antimicrobial drugs [18,19].

The aim of this study was to evaluate the application of different laser wavelengths with or without three photosensitizing dyes, erythrosine, curcumin and toluidine blue, on *Candida albicans* cells *in vitro* and in APDT protocols *in vivo* in experimentally infected larvae of *Galleria mellonella*.

2 Materials and methods

2.1 Microbial strain and culture conditions

The reference *C. albicans* strain SC5314 used in this study was grown on Sabouraud dextrose agar (SDA) plates at 30 °C for 24 hours. Cells from isolated colonies were suspended in 199 medium and incubated for 1 h at 37 °C with shaking, then collected by centrifugation and washed once with water. Yeast cells were counted using a Burker hemocytometer (Emergo, Landsmeer, The Netherlands) and the yeast cell suspension was properly diluted in sterile distilled water to achieve a final concentration of 5×10^7 cells/ml.

2.2 Dyes and laser sources

Erythrosine (Sigma Aldrich S.r.l., Milan, Italy, dye content >80%) and toluidine blue (Sigma Aldrich, dye content ~80%) were dissolved in distilled sterile water and curcumin (Sigma Aldrich, dye content ≤100%) in dimethyl sulfoxide (DMSO) to obtain 20 mM stock solutions. The final working concentration was 100 µM for erythrosine and curcumin, and 10 µM for toluidine blue.

Three laser prototypes, whose optical power was measured with a power meter (PM-200, Thorlabs), were used for this study: a red diode laser (spot size 0.2 cm^2), wavelength 650 nm, to be coupled with toluidine blue; a blue-violet diode laser (spot size 0.2 cm^2), wavelength 405 nm, to be coupled with curcumin; a green diode laser (spot size 0.785 cm^2), wavelength 532 nm, to be coupled with erythrosine.

Laser irradiation has been performed in continuous mode for the different wavelengths. Time irradiation was planned for each wavelength for fluences of 10 J/cm^2, 20 J/cm^2, and 30 J/cm^2 on the basis of the recorded power.

2.3 *In vitro* studies

Yeast cells were seeded on solid medium (SDA with or without dyes) or suspended in 20 µl of sterile distilled water (with or without dyes) in Eppendorf tubes. The plates were irradiated according to the 3 selected fluence

values in a half of the plate, while the second half served as a control, as described in a previous study [20]. For fungal suspensions, each parameter has been tested individually. Non irradiated fungal suspensions were used as growth control. All conditions have been tested in duplicate.

Overall, for each experimental condition were prepared 4 agar plates, 2 with and 2 without dye, and 16 Eppendorf tubes, 8 with and 8 without dye.

650 nm diode laser was applied for 307, 615 and 923 s to plates and Eppendorf tubes with and without toluidine blue; 405 nm diode laser was applied for 50, 100 and 150 s to plates and Eppendorf tubes with and without curcumin; 532 nm diode laser was applied for 95, 190 and 285 s to plates and Eppendorf tubes with and without erythrosine.

After irradiation, agar plates were incubated at 37 °C in aerobic conditions and observed after 1 day for the presence of growth inhibition in the irradiated area.

The content of each irradiated and non-irradiated Eppendorf tube (20 µl) was streaked on the entire surface of SDA plates. After 2 days of incubation at 37 °C in aerobic conditions colonies were enumerated. The antifungal effect was evaluated as percentual reduction of colony forming units (CFU) compared to non irradiated suspensions.

2.4 *Galleria mellonella* model

Larvae of *G. mellonella* at their final instar stage, selected for their weight (350 ± 20 mg) and the absence of cuticle pigmentation, were randomly divided into 14 groups (16 larvae/group) to evaluate the lack of toxicity of dyes and APDT efficacy after infection with *C. albicans*. An additional group consisting of untouched larvae served as a control for general viability.

In a first step, to evaluate the lack of toxicity of dyes, 10 µl of saline solution or dyes at the selected concentrations were inoculated directly into the hemocoel, via the last left pro-leg, in 4 groups of larvae.

For evaluation of APDT efficacy, 10 µl of a *C. albicans* SC5314 suspension (5×10^5 cells/larva) in saline solution (4 groups) or dyes (2 groups for each dye) were inoculated.

The irradiation for the *in vivo* model on *G. mellonella* has been performed in continuous mode for the different wavelengths at 10 J/cm^2. Immediately after infection, red diode laser, 650 nm, was applied for 307 s to each larva of 2 groups (infected in saline solution and infected in toluidine blue solution); blue diode laser, 405 nm, was applied for 50 s to each larva of 2 groups (infected in saline solution and infected in curcumin solution); green diode laser, 532 nm, was applied for 95 s to each larva of 2 groups (infected in saline solution and infected in erythrosine solution). Infected untreated larvae served as the control group.

Larvae were then transferred into clean Petri dishes, one for each experimental group, incubated at 37 °C in the dark for 9 days, and scored daily for survival.

Survival curves of treated and control animals were compared by the Mantel-Cox log-rank test using GraphPad Prism 5 software. A p value <0.05 was considered statistically significant.

All experiments were repeated twice, representative results are presented.

3 Results

No inhibition of *C. albicans* growth was observed after laser application on solid medium without dye. An area of growth inhibition was visible in agar plates added with curcumin and erythrosine, after application of blue diode laser and green diode laser, respectively. The areas of inhibition for the fluences of 10, 20 and 30 J/cm^2 had a diameter of 6.38 ± 0.6 mm, 8.51 ± 0 mm and 8.51 ± 0 mm, respectively, on the plates with curcumin, and of 10.2 ± 0 mm, 11.9 ± 0 mm and 11.9 ± 0 mm, respectively, on the plates with erythrosine.

Results of *in vitro* studies on fungal suspensions are summarized in Tables 1–3. No growth inhibition was obtained with all laser applications at any fluence value on fungal suspensions in the absence of dyes. Red diode laser coupled with toluidine blue caused a growth inhibition variable between 79.31%, for fluence of 10 J/cm^2, and 95.79% for fluences of 20 and 30 J/cm^2. The maximum inhibition of growth was obtained coupling blue diode laser and curcumin, with 100% of growth inhibition at any fluence. Green diode laser coupled with erythrosine caused a growth inhibition variable between 10.34%, for fluence of 10 J/cm^2, and 39.85% for fluence of 30 J/cm^2, but these values were not statistically significant.

Table 1 Effect of red laser (650 nm) application on *Candida albicans* suspensions with and without toluidine blue.

alt-text: Table 1

Sample	Mean CFU	SD	% inhibition *vs* control
Control	261	5	–
Red diode without toluidine blue	331	2	−26.82

	Fluence 10 J/cm²			
	Fluence 20 J/cm²	307	40	−17.62
	Fluence 30 J/cm²	311	2	−19.16
Toluidine blue 10 µM		62	13	76.25**
Red diode with toluidine blue	Fluence 10 J/cm²	54	74	79.31*
	Fluence 20 J/cm²	11	13	95.79***
	Fluence 30 J/cm²	11	13	95.79***

CFU, colony forming units; SD, standard deviation.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ *vs* control assessed by Student's *t*-test.

Table 2 Effect of blue laser (405 nm) application on *Candida albicans* suspensions with and without curcumin.

alt-text: Table 2

Sample		Mean CFU	SD	% inhibition *vs* control
Control		261	5	–
Blue diode without curcumin	Fluence 10 J/cm²	262	11	−0.38
	Fluence 20 J/cm²	255	5	2.30
	Fluence 30 J/cm²	259	20	0.77
Curcumin 100 µM		219	11	16.09*
Blue diode with curcumin	Fluence 10 J/cm²	0	0	100***
	Fluence 20 J/cm²	0	0	100***
	Fluence 30 J/cm²	0	0	100***

CFU, colony forming units; SD, standard deviation.

*$p < 0.05$; ***$p < 0.001$ *vs* control assessed by Student's *t*-test.

Table 3 Effect of green laser (532 nm) application on *Candida albicans* suspensions with and without erythrosine.

alt-text: Table 3

Samples		Mean CFU	SD	% inhibition *vs* control
Control		261	5	–
Green diode without erythrosine	Fluence 10 J/cm²	259	50	0.77
	Fluence 20 J/cm²	292	23	−11.88
	Fluence 30 J/cm²	287	12	−9.96
Erythrosine100 µM		305	49	−16.86

Green diode with erythrosine	Fluence 10 J/cm²	234	52	10.34
	Fluence 20 J/cm²	237	21	9.20
	Fluence 30 J/cm²	157	67	39.85

CFU, colony forming units; SD, standard deviation.

There was no significant difference in survival between larvae inoculated with saline (control group), or with dyes (Fig. 1), thus assessing their lack of toxicity in this experimental model at the adopted conditions.

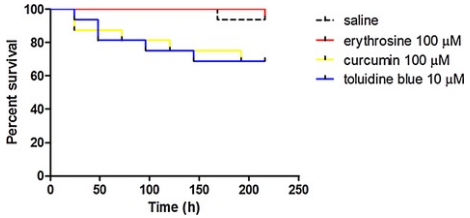

Fig. 1 Survival curves of *G. mellonella* larvae injected with saline solution or dyes (16 animals/group). No significant difference in survival was observed between the 4 groups.

alt-text: Fig. 1

In larvae infected with *C. albicans* SC5314, the every performed treatment (laser alone, dye alone or combinations of laser and dye) led to a significant increase in survival in comparison to untreated animals inoculated with *C. albicans* cells in saline ($p < 0.001$) (Figs. 2–4).

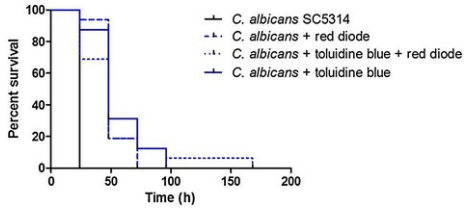

Fig. 2 Survival curves of *G. mellonella* larvae infected with *C. albicans* (5×10^5 cells/larva) in saline or toluidine blue (16 animals/group), exposed or not to irradiation with red diode laser (650 nm). A significant difference ($p < 0.001$) was observed in survival between the control group (*C. albicans* alone) and the other three groups, as assessed by the Mantel-Cox log-rank test. No significant difference in survival was observed between the 3 groups of larvae infected with toluidine blue, irradiated or not with red diode laser, and infected with *C. albicans* without toluidine blue and irradiated with red diode laser. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

alt-text: Fig. 2

The combination of toluidine blue and red diode laser application led to a prolonged survival compared to dye alone or laser application alone, although the difference in survival was not statistically significant between the 3 groups (Fig. 2). A statistically significant difference ($p = 0.02$) in survival was found between the group inoculated with curcumin alone compared to the group treated with blue diode laser coupled with curcumin (Fig. 3).

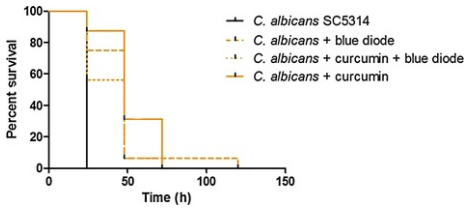

Fig. 3 Survival curves of *G. mellonella* larvae infected with *C. albicans* (5×10^5 cells/larva) in saline or curcumin (16 animals/group), exposed or not to irradiation with blue diode laser (405 nm). A significant difference ($p < 0.001$) was observed in survival

between the control group (*C. albicans* alone) and the other three groups, as assessed by the Mantel-Cox log-rank test. A statistically significant difference ($p = 0.02$) was found also between the group of larvae infected with curcumin in comparison to the group infected with curcumin and treated with blue diode laser. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

alt-text: Fig. 3

A statistically significant difference ($p = 0.03$) in survival was found between the group of larvae irradiated with green diode laser compared to the group treated with laser coupled with erythrosine (Fig. 4).

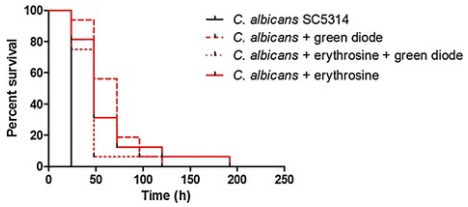

Fig. 4 Survival curves of *G. mellonella* larvae infected with *C. albicans* (5×10^5 cells/larva) in saline or erythrosine (16 animals/group), exposed or not to irradiation with green diode laser (532 nm). A significant difference ($p < 0.001$) was observed in survival between the control group (*C. albicans* alone) and the other three groups, as assessed by the Mantel-Cox log-rank test. A statistically significant difference ($p = 0.03$) was found also between the group of infected larvae irradiated with green diode laser in comparison to the group infected with erythrosine and treated with green diode laser. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

alt-text: Fig. 4

4 Discussion

In this study, three different wavelengths were applied with or without photosensitizers on *C. albicans* cells *in vitro*. No growth inhibition was observed without photosensitizers in both liquid and solid medium assays.

The maximum inhibition of yeast growth was obtained coupling 405 nm diode laser application and curcumin, with 100% of growth inhibition on fungal suspensions at any fluence, a result confirmed by the presence of inhibition areas on solid medium, while laser alone had no effect. Curcumin is described as 2.5 fold more potent than fluconazole at inhibiting the adhesion of *C. albicans* to buccal epithelial cells [21]. It has been reported to generate reactive oxygen species and trigger an early apoptosis in *C. albicans* cells and to cause membrane disruption [22,23] The antifungal properties of curcumin seem to be improved by the action of laser light and the maximum effect is achieved at the lowest fluence value.

Toluidine blue alone caused a consistent reduction of *C. albicans* cells in suspension in comparison to control. 650 nm diode laser coupled with toluidine blue caused a growth inhibition on fungal suspensions variable between 79.31% and 95.79%, while no inhibition was observed on solid medium. Conversely, 532 nm diode laser used with erythrosine led to a slight, not significant growth inhibition on fungal suspensions, but caused the appearance of large inhibition areas on solid medium. The explanation for these apparently contradictory results may be related to some differential reaction of the dyes in liquid and solid medium: this may be the subject for future studies aiming to clarify the mechanism of photodynamic inactivation against *C. albicans*.

The *in vivo* study exploited the recently adopted non-vertebrate *G. mellonella* model to explore the efficacy of laser APDT against C. *albicans* infection. This host model provides many competitive advantages over mammalian models, such as an important reduction in time, cost and ethical or legal concerns, other than simplicity to treat (e.g. without anaesthesia) and maintain a large number of animals.

For every treatment applied (laser alone, dye alone or combination of dye and laser), we obtained a significant difference in survival curves in comparison to the control group. It has been previously proven that no one of the used dyes was toxic for *G. mellonella* in the adopted experimental conditions.

Infected larvae inoculated with erythrosine showed a prolonged survival curve in comparison to those inoculated with erythrosine and treated with 532 nm diode laser, although a statistically significant difference ($p = 0.03$) in survival was found only between the group of larvae irradiated with 532 nm diode laser in respect to the group treated with laser coupled to erythrosine. One hypothesis for this result may be that the combination of laser and erythrosine could create some alterations in the dye molecule decreasing the candidacidal effect. A similar hypothesis could be formulated for blue laser and curcumin, where laser application alone resulted in a prolonged survival, but a statistically significant difference ($p = 0.02$) was found only between the group inoculated with curcumin in comparison to the group inoculated with curcumin and irradiated with 405 nm diode laser. These results are comparable with those of Carmello et al., who reported better results for blue light alone rather than in combination with curcumin [24].

Instead, the combination of toluidine blue and 650 nm diode laser application led to a prolonged survival compared to the groups inoculated with dye alone or treated with laser application alone, although the

difference in survival was not statistically significant.

The insect immune response demonstrates structural and functional similarities to the innate immune response of mammals and, in particular, insect haemocytes and mammalian neutrophils have been shown to phagocytose and kill pathogens in a similar manner. Recent studies demonstrated that APDT can stimulate host defense mechanisms via the attraction and accumulation of neutrophils into the infected region [19]. This may explain, at least in part, the results obtained in *G. mellonella*.

Other studies will be necessary to validate these results, and to better understand the interaction among the host immune system, *C. albicans*, photosensitizers and lasers. APDT protocols could be also evaluated versus or in combination with antifungals.

APDT is reported in literature as effective against bacteria resistant to antibiotics and not inducing the creation of resistant strains; in fact, the resistance to this treatment is unlikely, given that singlet oxygen and free radicals interact with different cell structures and different metabolic pathways [25–30].

Moreover the presence of several and different cellular targets in fungi may reduce the risk of selection of photomutant resistant strains with a further reduction for this risk thanks to the lack of mutagenic effect for PDT [31].

Moreover, PDT is low cost and possesses low overdose risk.

Overall, APDT does represent an actual alternative for treatment of fungal infections, since the topical administration of a photosensitizer followed by laser application is a simple process without systemic toxicity.

Conflict of interest

Authors disclosed no potential financial conflict of interest with this study.

~~Appendix ASupplementary dataSupplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.pdpdt.2017.01.181.Appendix ASupplementary dataThe following are Supplementary data to this article:~~References

[1] C. Spagnul, L.C. Turner and R.W. Boyle, Immobilized photosensitizers for antimicrobial applications, *J. Photochem. Photobiol. B* **150**, 2015, 11–30.

[2] D.F. Dolmans, D. Fukumura and R.K. Jain, Photodynamic therapy for cancer, *Nat. Rev. Cancer* **3**, 2003, 380–387.

[3] K. Konopka and T. Goslinski, Photodynamic ~~Therapy in D~~therapy in dentistry, *J. Dent. Res.* **86**, 2007, 694–707.

[4] M.C.A. Issa and M. Manela-Azulay, Photodynamic Therapy: a review of the literature and image documentation, *An. Bras. Dermatol.* **85**, 2010, 501–511.

[5] R.R. Allison, G.H. Downie, R. Cuenca, X.H. Hu, C.J. Childs and C.H. Sibata, Photosensitizers in clinical PDT, *Photodiagn~~osis~~. Photodyn. Ther.* **1**, 2004, 27–42.

[6] P. Meisel and T. Kocher, Photodinamic therapy for periodontal diseases: state of the art, *J. Photochem. Photobiol. B* **79**, 2005, 159–170.

[7] K.A. Salva, Photodynamic Therapy: unapproved uses, dosages, or indication, *Clin. Dermatol.* **20**, 2002, 571–581.

[8] A.C. Kübler, Photodynamic therapy, *Med. Laser Appl.* **20**, 2005, 37–45.

[9] R.R. Allison, H.C. Mota and C.H. Sibata, Clinical PD/PDT in ~~North America: A~~north america: an historical review, *Photodiagn~~osis.~~ Photodyn. Ther.* **1**, 2004, 263–277.

[10] A. Juzeniene, P. Juzenas, L.W. Ma, V. Iani and J. Moan, Effectiveness of different light sources for 5-aminolevulinic acid photodynamic therapy, *Lasers Med. Sci.* **19**, 2004, 139–149.

[11] A. Pieslinger, K. Plaetzer, C.B. Oberdanner, J. Berlanda, H. Mair, B. Krammer and T. Kiesslich, Characterization of a simple and homogenous irradiation device based on light-emitting diodes: a possible low-cost supplement to conventional light sources for photodynamic treatment, *Med. Laser Appl.* **21**, 2006, 277–283.

[12] R. Steiner, New laser technology and future applications, *Med. Laser Appl.* **21**, 2006, 131–140.

[13] T.G. St Denis, T. Dai, L. Izikson, C. Astrakas, R.R. Anderson, M.R. Hamblin and G.P. Tegos, All you need is light: antimicrobial photoinactivation as an evolving and emerging discovery strategy against infectious disease, *Virulence* **2**, 2011, 509–520.

[14] R.F. Donnelly, P.A. McCarron and M.M. Tunney, Antifungal photodynamic therapy, *Microbiol. Res.* **163**, 2008, 1–12.

[15] J.P. Lyon, L.M. Moreira, P.C. de Moraes, F.V. dos Santos and M.A. de Resende, Photodynamic therapy for pathogenic fungi, *Mycoses* **54**, 2011, e265–271.

[16] F. Javed, L.P. Samaranayake and G.E. Romanos, Treatment of oral fungal infections using antimicrobial photodynamic therapy: a systematic review of currently available evidence, *Photochem. Photobiol. Sci.* **13**, 2014, 726–734.

[17] Y.I. Liang, L.M. Lu, Y. Chen and Y.K. Lin, Photodynamic therapy as an antifungal treatment, *Exp. Ther. Med.* **12**, 2016, 23–27.

[18] J. Chibebe Junior, B.B. Fuchs, C.P. Sabino, J.C. Junqueira, A.O.C. Jorge, M.S. Ribeiro, M.S. Gilmore, L.B. Rice, G.P. Tegos, M.R. Hamblin and E. Mylonakis, Photodynamic and antibiotic therapy impair the pathogenesis of *Enterococcus faecium* in a whole animal insect model, *PLoS One* **8**, 2013, e55926.

[19] J. Chibebe Junior, C.P. Sabino, X. Tan, J.C. Junqueira, Y. Wang, B.B. Fuchs, A.O. Jorge, G.P. Tegos, M.R. Hamblin and E. Mylonakis, Selective photoinactivation of *Candida albicans* in the non-vertebrate host infection model *Galleria mellonella*, *BMC Microbiol.* **13**, 2013, 217.

[20] P. Vescovi, S. Conti, E. Merigo, T. Ciociola, L. Polonelli, M. Manfredi, M. Meleti, C. Fornaini, J.P. Rocca and S.A. Nammour, In vitro bactericidal effect of Nd:YAG laser on *Actinomyces israelii*, *Lasers Med. Sci.* **28**, 2013, 1131–1135.

[21] C.V. Martins, D.L. da Silva, A.T. Neres, T.F. Magalhães, G.A. Watanabe, L.V. Modolo, A.A. Sabino, A. de Fátima and M.A. de Resende, Curcumin as a promising antifungal of clinical interest, *J. Antimicrob. Chemother.* **63**, 2009, 337–339.

[22] M. Sharma, R. Manoharlal, N. Puri and R. Prasad, Antifungal curcumin induces reactive oxygen species and triggers an early apoptosis but prevents hyphae development by targeting the global repressor TUP1 in *Candida albicans*, *Biosci. Rep.* **30**, 2010, 391–404.

[23] W. Lee and D.G. Lee, An antifungal mechanism of curcumin lies in membrane-targeted action within *Candida albicans*, *IUBMB Life* **66**, 2014, 780–785.

[24] J.C. Carmello, A.C. Pavarina, R. Oliveira and B. Johansson, Genotoxic effect of photodynamic therapy mediated by curcumin on *Candida albicans*, *FEMS Yeast Res.* **15**, 2015, fov018.

[25] T. Dai, Y.Y. Huang and M.R. Hamblin, Photodynamic therapy for localized infections–state of the art, *Photodiagnosis. Photodyn. Ther.* **6**, 2009, 170–188.

[26] M. Wainwright and K.B. Crossley, Photosensitizing agents-circumventing resistance and breaking down biofilms: a review, *Int. Biodeterior. Biodegrad.* **53**, 2004, 119–126.

[27] M.R. Hamblin and T. Hasan, Photodynamic therapy: a new antimicrobial approach to infections disease?, *Photochem Photochem. Photobiol. Sci.* **3**, 2004, 436–450.

[28] K. O'Riodan, O.E. Akilov and T. Hasan, The potential for photodynamic therapy in the treatment of localized infections, *Photodiagnosis. Photodyn. Ther.* **2**, 2005, 247–262.

[29] S. Silverman, Oral Cancer: complications of therapy, *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **88**, 1999, 122–126.

[30] N. Komerik and A.J. MacRobert, Photodynamic therapy as an alternative antimicrobial modality for oral infections, *J. Environ. Pathol. Toxicol. Oncol.* **25**, 2006, 487–504.

[31] P.G. Calzavara-Pinton, M. Venturini and R. Sala, A comprehensive overview of photodynamic therapy in the treatment of superficial fungal infections of the skin, *J. Photochem. Photobiol. B* **78**, 2005, 1–6.

Highlights

- The model *G. mellonella* to explore laser PDT provides advantages over mammalian models.
- PDT may be a good alternative to antimicrobial drugs especially for the treatment of localized infections.
- PDT does not generate fungal resistance.
- PDT is of low cost and possesses low overdose risk.
- PDT is a simple process without systemic toxicity.

Queries and Answers

Query: The author names have been tagged as given names and surnames (surnames are highlighted in teal color). Please confirm if they have been identified correctly.
Answer: Yes

Query: “Your article is registered as a regular item and is being processed for inclusion in a regular issue of the journal. If this is NOT correct and your article belongs to a Special Issue/Collection please contact e.rajesh@elsevier.com immediately prior to returning your corrections.”
Answer: Article for regular issue

Query: Figs. 2,3,4 will appear in black and white in print and in color on the web. Based on this, the respective figure captions have been updated. Please check, and correct if necessary.
Answer: ok

Query: Please check the presentation all of the Tables and correct if necessary.
Answer: ok