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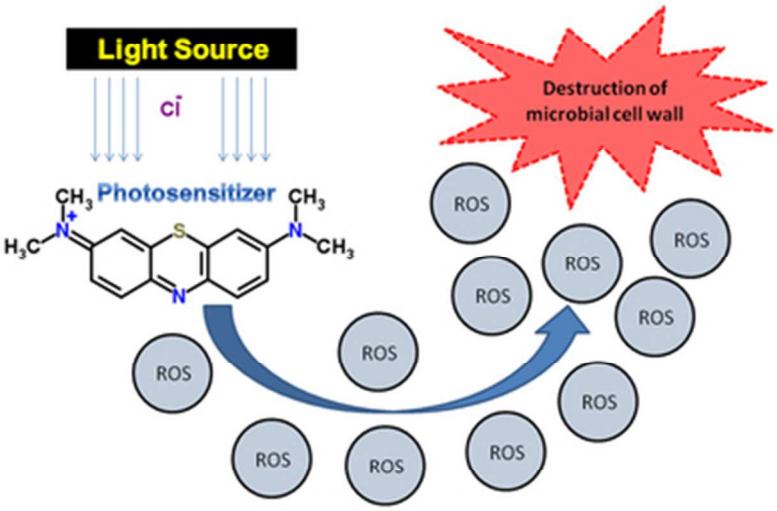

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Title: Treatment of oral fungal infections using antimicrobial photodynamic therapy: A systematic review of currently available evidence

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Running title: Oral fungal infections and PDT

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ABSTRACT

The aim was to review the efficacy of antimicrobial photodynamic therapy (PDT) in the treatment of oral fungal infections. To address the focused question "Should PDT be considered a possible treatment regimen for oral fungal infections?" PubMed/Medline and Google-Scholar databases were searched from 1997 up to March 2014 using various combinations of the following key words: "*Candida albicans*; "Candidiasis"; "Candidosis"; "denture stomatitis"; "oral" and "photodynamic therapy". Original studies, experimental studies and articles published solely in English language were sought. Letters to the editor, historic reviews and unpublished data were excluded. Pattern of the present literature review was customized to mainly summarize the pertinent information. Fifteen studies (3 clinical and 12 experimental) were included. All studies reported antimicrobial PDT to be an effective antifungal treatment strategy. One study reported PDT and azole therapy to be equally effective in the treatment of oral fungal infections. Methylene blue, toluidine blue and porphyrin derivative were the most commonly used photosensitizers. The laser wavelengths and power output ranged between ~455nm-660nm and 30mW-400mW. The energy fluence ranged between 26-245 J/cm^2 and the duration or irradiation ranged between 10 seconds and 26 minutes. Clinical effectiveness of antimicrobial PDT as a potent therapeutic strategy for oral fungal infections requires further investigations.

Key words: *Candida albicans*; Candidiasis; Candidosis; denture stomatitis; oral and photodynamic therapy.

1. Introduction

Candida species (predominantly *Candida albicans* [*C. albicans*]) are components of the normal oral flora [1-3]; however, *C. albicans* is also the most common etiological agent associated with oral fungal infections (such as oral candidiasis and denture stomatitis [DS]) and corresponds to nearly 80% of all microorganisms isolated from oral lesions [4, 5]. The conventional treatment of oral fungal infections is associated with a precise diagnosis, identification and elimination of possible risk factors (such as tobacco habits [6], prolonged use of corticosteroids and antibiotics [7-9] and poor oral and denture hygiene [9-11]) and prescription of either topical or systemic antifungal agents [12-14]. However, host toxicity and potential to jeopardize and interrupt cellular function are major limitations of antifungal drugs [15]. Furthermore, another challenge posed to clinicians is the resistance of *Candida* species (primarily *C. albicans*) to antifungal agents by the expression of efflux pumps that reduce drug accumulation and alter the structure and concentration of antifungal target proteins and membrane sterol composition [16].

Photodynamic therapy (PDT) is a modern therapeutic strategy that involves interactions between a light source of a particular wavelength and a photosensitizer (PS) in the presence of oxygen [17-19]. This phototoxic and chemical reaction induces the production of reactive oxygen species (ROS) that cause oxidative damage to the target cells including microbial cells and tumor cells [20, 21]. Briefly, perks of PDT encompass the following: (a) high target specificity [22]; (b) biocompatibility with healthy human cells [23]; (c) unlikely risk of chemical and/or thermal side-effects [24]; and (d) improbable chances of microbes to develop resistance against PDT [15].

Since *C. albicans*, a significant contributor to the etiology of oral fungal infections (such as candidiasis and denture stomatitis), has demonstrated resistance to traditional antifungal drugs (such as azoles) [25]; it is speculated that PDT is a modern and more promising therapeutic strategy for the treatment of oral fungal infections compared to traditional antifungal drug therapy. In this regard, the aim of the present study was to systematically review the pertinent literature with reference to the susceptibility of oral candidiasis to antimicrobial PDT.

2. Materials and methods

2.1. Focused question

The addressed focused question was "Should PDT be considered a possible treatment regimen for oral fungal infections?"

2.2. Eligibility criteria

Eligibility criteria comprised of the following: 1) Original studies; 2) Experimental studies; 3) Clinical studies; 4) Reference list of potentially relevant original and review articles; 5) Intervention: treatment of oral fungal infections using antimicrobial PDT; and 6) Studies published solely in English-language. Case-reports, letters to the editor, historic reviews and unpublished data were excluded (Figure 1).

2.3. Search strategy

MEDLINE/PubMed (National Library of Medicine, Bethesda, Maryland) and Google-Scholar databases were searched from 1997 up to and including March 2014

using the following terms in different combinations: "*Candida albicans*; "Candidiasis"; "Candidosis"; "denture stomatitis"; "oral" and "photodynamic therapy". As a next step, titles and abstracts of studies that fulfilled the eligibility criteria were screened and checked for agreement. Reference lists of original and review articles that were found to be relevant were hand searched. Due to heterogeneity of the studies, a meta-analysis could not be performed (Figure 1).

The initial search yielded 19 studies. Scrutiny of the titles and abstracts condensed the number of included studies to 15 [26-40]. Four studies that did not abide by the eligibility criteria were excluded (Appendix A). Since a limited numbers of original studies addressed our focused question, the pattern of the present systematic review was customized to primarily summarize the pertinent data.

3. Results

3.1. Characteristics of included studies

In total, 15 studies [26-40] were included which were performed at either universities or healthcare centers. Three studies [27, 28, 39] were clinical and 12 studies [26, 29-38, 40] had an experimental research design. Two clinical studies [27, 39] focused on treatment of DS using PDT whereas in one clinical study [28], antifungal effects of PDT and Fluconazole were compared in 21 HIV-positive patients with oral candidiasis.

Scwingel et al. [28] reported that PDT is more effective in the treatment of oral candidiasis compared to traditional antifungal therapy using azoles. Regarding the treatment of patients with DS, Mima et al. [27] reported that PDT and

conventional antifungal drug therapy (Nystatin [NYT]) are equally effective in the treatment of DS; whereas another clinical study [39] reported PDT to clinically reduce inflammation on palatal mucosa.

Amongst the 12 experimental studies [26, 29-38, 40] included in the present review, six studies [26, 29, 33, 35, 37, 38] were performed on animal models, five studies [30-32, 34, 36] were performed on cultured oral *Candida* isolates and one study [40] was performed on denture models. All experimental studies [26, 29-38, 40] reported PDT to be an effective antifungal therapy.

3.2. Characteristics of lasers used in photodynamic therapy

In all 15 studies [26-40], diode lasers with wavelengths ranging between ~455 nm and 660 nanometers (nm) were used with power output ranging from 30 milliwatts (mW) to 400 mW. Six studies [28-32, 36] reported the surface area exposed to laser irradiation, which ranged between 0.04 square centimeters (cm^2) and 1.13 cm^2. Fourteen studies [26-37, 39, 40] reported the energy fluence of the diode lasers, which ranged from 26 joules per square centimeters (J/cm^2) up to 245 J/cm^2. Power density of the lasers was reported in six studies [26, 27, 32, 34, 39] that ranged between 24 milliwatts per square centimeters (mW/cm^2) and 526 mW/cm^2. In 14 studies [26-33, 35-40], duration of irradiation ranged between ~0.2 minutes and 26 minutes (Table 2).

Two clinical studies [27, 39] that reported PDT to be effective in eliminating oral *Candida* from denture surfaces used diode lasers with a wavelength, power density and energy fluence of 455 nm, 24 mW/cm^2 and 37.5 J/cm^2, respectively and the duration of denture surface irradiation was 26 minutes. In these studies [27, 39]

the palatal mucosa was also treated by PDT using diode lasers with a wavelength, power density and energy fluence of 455 nm, 102 mW/cm^2 and 122 J/cm^2, respectively. In both studies [27, 39], the palatal mucosae were irradiated for 20 minutes. In their study on humans, Scwingel et al. [28] reported PDT to be an effective therapy for oral candidiasis, a diode laser with wavelength, energy fluence and power output of 660 nm, 7.5 122 J/cm^2, 30 mW, respectively was used. The target oral tissues were 0.04 cm^2 in area and the duration of irradiation was 10 seconds (~0.2 minutes) [28] (Table 2).

Among the experimental studies [26, 29-38, 40], laser wavelength, energy fluence, power output, power density and duration of irradiation ranged between ~455 nm to 664 nm, 37.5 J/cm^2 to 245 J/cm^2, 40 mW to 200 mW and 3 minutes to 26 minutes correspondingly (Table 2).

3.3. Characteristics of photosensitizers used in photodynamic therapy

In two clinical [27, 34] and three experimental studies [35, 39, 40], porphyrin derivatives were used as PS whereas methylene blue (MB), toluidine blue (TBO) or both were used as PS in one clinical [28] and five experimental studies [30, 33, 36-38]. Dovigo et al. [26] and Costa et al. [31] used curcumin and erythrosine, respectively as PS; whereas in one study [32], rose-bengal and erythrosine were used as PS. The pre-irradiation time ranged between 1 minute and 1440 minutes. TBO was used at a concentration of 10 milligrams per deciliter (mg/dL) in two studies [30, 36]. In five experimental studies [30, 33, 36-38] 10 mg/dL MB was used as PS during PDT; whereas in their study on humans, Scwingel et al. [28] used 45 mg/dL MB as PS during PDT to treat oral candidiasis. In five studies [27, 34, 35, 39, 40], porphyrin derivate was used as PS at concentrations ranging between 2.5 mg/dL and 100 mg/dL.

Dovigo et al. [26] treated experimental candidiasis via PDT using curcumin (as PS) at three concentrations (0.74 mg/dL, 1.47 mg/dL and 2.95 mg/dL) with a pre-irradiation time of 20 minutes; whereas in two experimental studies [29, 31] erythrosine was used as PS at concentrations of 4.5 mg/dL and 12 mg/dL respectively. In these studies [29, 31] the pre-irradiation time was one minute and 5 minutes, respectively (Table 3).

3.4. Drug (photosensitizer) Delivery

Among the studies based on humans [27, 28, 39] and animal models [29, 33, 35, 37, 38], photosensitizers were topically applied on affected areas (including dorsum of tongue and palatal surfaces). In studies by Mima et al. [27, 39, 40] denture surfaces were sprayed with photosensitizers prior to light application. Among the in-vitro studies [30, 31, 32, 34, 36], photosensitizers were placed in 96-well plates and exposed to light (Table 3).

4. Discussion

Results from virtually 93% of the studies [26, 28-40] that fulfilled our eligibility criteria demonstrated that antimicrobial PDT is an efficient therapeutic strategy in treating oral fungal infections. Although results from the studies [26-40] that fulfilled our eligibility criteria appeared persuasive enough to conclude that PDT exhibits antifungal effects (even against azole-resistant fungi); we observed an inconsistency in the laser parameters and concentration/type of PS used in these studies [26-40]. For example, experimental studies by Pupo et al. [30], Costa et al. [32] and Martins Jda et al. [33] reported PDT to exhibit antifungal effects against *C. albicans.* In these studies [30, 32, 33] although the laser parameters (660 nm, 475 nm and 660 nm, respectively) were comparable; the energy fluence (53 J/cm^2, 95 J/cm^2

and up to 245 J/cm^2) and power output (40 mW, 200 mW and 100 mW) were inconsistent. In addition, parameters such as irradiated area and power density of the laser were reported by only a limited number of studies (six studies reported the irradiated area [28-32, 36] and five studies [26, 32, 34, 39, 40] reported the power density. Furthermore, the duration of irradiation also varied among the studies (from 10 seconds up to 26 minutes). Since most of the laser parameters varied between studies included in this review, it is arduous to accurately pinpoint the parameters that would be most effective in treating oral fungal infections. It is however worth mentioning that two studies [27, 39], which focused on the treatment of DS via PDT showed consistency in laser parameters to an extent. However, further clinical studies on the treatment of DS using PDT are warranted to reach a consensus over the precise laser parameters that could completely eradicate *Candida* species from denture and palatal surfaces.

It has been reported that concentration of PS used during PDT affects the overall antimicrobial efficacy of PDT [41]. Among the studies that reported antimicrobial PDT to be effective in eliminating oral *Candida* species, 40% studies used either MB or TBO or both as PS [28, 30, 33, 36-38], nearly 33% studies [27, 35, 39, 40] used hematoporphyrin derivative as PS and approximately 26% studies used either curcumin [26], erythrosine [29, 31], rose-bengal [32] or malachite green [36] as PS. However, from the literature reviewed, we observed an inconsistency in the concentration of the PS used during antimicrobial PDT. For example, in experimental studies [30, 33, 36-38], MB was used at a concentration of 10 mg/dL whereas in a study on humans [28], the same photosensitizer was used at a concentration of 45 mg/dL. Likewise, experimental studies by Mang et al. [34] and Mima et al. [35] used porphyrin derivative at varying concentrations (2.5 mg/dL and 40 mg/dL/50

mg/dL/100 mg/dL, respectively). With reference to the clinical results by Mima et al. [27] it is speculated that porphyrin derivate when used during PDT at a concentration of 50 mg/dL is effective in the treatment of DS. However, due to a lack of sufficient clinical evidence, it is challenging to standardize PS concentrations that should be used for antifungal therapy via PDT. To stretch the argument further, it is pondered that in a clinical setting, the concentration of PS used may vary according to the severity of the oral fungal infection. To our knowledge, efficacy of antimicrobial PDT with reference to disease severity remains uninvestigated. Further randomized controlled clinical trials are warranted to standardize the concentrations of PS that would significantly reduce oral *Candida* in patients with oral fungal infections.

We identified one clinical study [27] in which antimicrobial PDT and conventional azole drug therapy were reported to be equally effective in treating oral *Candida* infections. Although the beneficial outcomes of PDT reported by studies [26-40] included in the present review cannot be disregarded, it is pertinent to mention that ~87% studies [26, 29-40] included did not compare the antifungal efficacy of PDT with traditional fungicidal drug therapy. Hence, it remains unclear whether antimicrobial PDT is either as effective as conventional azole antifungal therapy or is superior in antifungal efficacy to the latter; however, some studies [30, 32, 33] cited in the present review reported that PDT exhibits antifungal effects even against azole-resistant fungi.

In the studies on humans [27, 28, 39] and animal models [29, 33, 35, 37, 38], photosensitizers were topically applied to infected sites. This is advantageous in the sense that the drug directly exposes the microbes to ROS following exposure to light as compared to systemic medication. Allergic reactions to photosensitizers (porphyrins) may rarely occur following PDT in dermal tissues [42]; there were no

allergic reactions reported in the oral cavity in any of the studies included in the present review. However, the possibility of hypersensitive reactions following topical application of photosensitizers on oral tissues cannot be disregarded.

It is well known that systemic conditions (such as poorly-controlled diabetes and prediabetes) and tobacco habits (such as cigarette smoking and tobacco chewing) influence oral *Candida* carriage [1-3, 43, 44]. In addition, it has also been hypothesized that tissue healing and repair are jeopardized in smokers and in patients with chronic hyperglycemia due to an increased expression of receptor of advanced glycation end products in the body tissues including oral cavity [45, 46]. Therefore, it is speculated that the efficacy of antimicrobial PDT is compromised in patients with poorly-controlled diabetes and among habitual tobacco users; however further studies are warranted in this regard.

5. Conclusion

On experimental grounds, PDT exhibits antimicrobial effects against oral *Candida*; however, the clinical effectiveness of antimicrobial PDT as a potent therapeutic strategy for oral fungal infections requires further investigations.

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Conflict of interest statement

None declared

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APPENDIX A. List of excluded studies. Reason for exclusion is shown in parenthesis.

a. N. S. Soukos, Goodson JM. Photodynamic therapy in the control of oral biofilms. *Periodontol. 2000*., 2011, **55**, 143-166 (Review article).
b. M. A. Biel, Photodynamic therapy of bacterial and fungal biofilm infections, *Methods. Mol. Biol*., 2010, **635**, 175-194 (Review article).
c. R. F. Donnelly, P. A. McCarron, M. M. Tunney and D. A. Woolfson. Potential of photodynamic therapy in treatment of fungal infections of the mouth. Design and characterization of a muco-adhesive patch containing toluidine blue O. *J. Photochem. Photobiol. B.*, 2007, **86**, 59-69 (Focused question not answered).
d. Y. Chabrier-Roselló, T. H. Foster, N. Pérez-Nazario, S. Mitra and C. G. Haidaris, Sensitivity of Candida albicans germ tubes and biofilms to photofrin-mediated phototoxicity, *Antimicrob. Agents. Chemother.*, 2005, **49**, 4288-4295 (Focused question not answered).

Table 1. Characteristics of studies that fulfilled our eligibility criteria

Authors et al.	Study design	Study subjects	Intervention	Study groups	Outcome
Dovigo et al.[26]	Experimental	40 immunosuppressed mice	Oral *C. albicans* inoculation	Test-group: PS+LED Positive control: No treatment Negative control: No *C. albicans* inoculation	Curcumin-mediated PDT was effective for *in-vivo* inactivation of C. albicans.
Mima et al. [27]	Clinical	40 patients	DS	Test-group: PS+LED Control-group: Topical NYT 4 times daily for 15 days	The test- and control groups showed clinical success rates of 45% and 53%. NYT and PDT were equally effective in the treatment of DS.
Scwingel et al. [28]	Clinical	21 HIV-positive patients	Oral Candidiasis	Control-group: Fluconazole 100mg/day during 14 days. Group-1: Laser alone Group-2: PDT	Antimicrobial PDT was effective in the treatment of oral candidiasis on HIV-positive patients.
Costa et al. [29]	Experimental	56 immunosuppressed mice	Oral *C. albicans* inoculation	Group-1: PDT on 48 sites with *C. albicans* Group-2: PDT on 8 sites without *C. albicans*	PDT exhibited antifungal effects against *C. albicans* biofilms.

Pupo et al. [30]	Experimental	Oral *Candida* suspensions in 96 well plates	Oral *C. albicans* inoculation	Group-1 (-ve Control): Saline Group-2 (+ve control): *C. albicans* + Saline Group-3: *C. albicans*+TBO Group-4: *C. albicans*+MB Group-5: *C. albicans*+Saline+laser Group-6: *C. albicans*+TBO+laser Group-7: *C. albicans*+MB+laser	PDT using either MB or TBO exhibited antifungal effects against *C. albicans* biofilms.
Costa et al. [31]	Experimental	Oral *Candida* suspensions in 96 well plates	Oral *C. albicans* inoculation	Test-group: PDT Control-group: PBS	PDT exhibited antifungal effects against *C. albicans* and *Candida dublinensis*.
Costa et al. [32]	Experimental	Oral *Candida* suspensions in 96 well plates	Oral *C. albicans* inoculation	Group-1: Rose-bengal+LED Group-2: Erythrosine+LED Group-3: No treatment	Erythrosine- and rose bengal-mediated PDT with LED irradiation were effective in treating *C. albicans*.
Martins Jda et al. [33]	Experimental	56 rats	Oral *C. albicans* inoculation	Group-1: No treatment Group-2: Laser alone Group-3: PS alone Group-4: PDT	PDT exhibited antifungal effects against *C. albicans*.
Mang et al. [34]	Experimental	Cultures of *Candida* strains derived from AIDS patients	Oral Candidiasis	Group-1: No treatment Group-2: Laser alone Group-3: PS alone Group-4: PDT	PDT exhibited antifungal effects against *C. albicans*.
Mima et al. [35]	Experimental	71 immunosuppressed mice	Oral *C. albicans* inoculation	Group-1: Light alone Group-2: PS alone Group-3: No treatment Group-3: PDT	PDT resulted in a significant reduction in *C. albicans* recovered from the tongue compared to other groups.

Souza et al. [36]	Experimental	Oral *Candida* suspensions in 96 well plates	Oral *C. albicans* inoculation	Group-1: Light alone Group-2: PS alone Group-3: No treatment Group-3: PDT	PDT is effective in the treatment of oral candidiasis.
Junqueira et al. [37]	Experimental	72 rats	Oral *C. albicans* inoculation	Group-1: Light alone Group-2: PS alone Group-3: No treatment Group-4: PDT	PDT is effective in the treatment of oral candidiasis.
Teichert et al. [38]	Experimental	75 immunosuppressed mice	Oral *C. albicans* inoculation	Group-1: Light alone Group-2: PS alone Group-3: No treatment Group-4: PDT	PDT can be potentially used in the treatment of oral candidiasis.
Mima et al. [39]	Clinical	5 patients	DS	Dentures and palates of all patients were treated with PDT	Four patients showed clinical resolution of DS after PDT and one patient demonstrated reduction in palatal inflammation. Recurrence of DS was observed in two patients.
Mima et al. [40]	Experimental	34 dentures	Candida growth on dentures	Group-1: Light alone Group-2: PS alone Group-3: PDT	PDT was effective in reducing *Candida* species On dentures

C. albicans: *Candida albicans* DS: Denture stomatitis LED: Light emitting diode MB: Methylene blue NYT: Nystatin
PBS: Phosphate buffered saline PS: Photosensitizer TBO: Toluidine Blue

Table 2. Laser parameters of studies that fulfilled our eligibility criteria.

Authors et al.	Laser parameters						
	Source	Wavelength (in nm)	Irradiated Area (in cm^2)	Energy fluence (in J/cm^2)	Power output (in mW)	Power density (in mW/cm^2)	Duration of irradiation (in minutes)
Dovigo et al.[26]	LED	~ 455	—	37.5	—	89.2	7
Mima et al. [27]	LED	455	—	Denture: 37.5 Palate: 122	260	Denture: 24 Palate: 102	Denture: 26 Palate: 20
Scwingel et al. [28]	LED	660	0.04	7.5	30	—	~0.2
Costa et al. [29]	Green LED	542	1.13	14.34	90	—	3
Pupo et al. [30]	InGaAlP	660	0.38	53	40	—	5
Costa et al. [31]	Green LED	542	0.38	42.63	90	—	3
Costa et al. [32]	Blue LED	475	0.38	95	200	526	3
Martins Jda et al. [33]	GaAlAs	660	—	up to 245	100	—	1.15
Mang et al. [34]	LED	630	—	up to 135	—	150	—
Mima et al. [35]	LED	455 or 630	—	305	200	—	20

Souza et al. [36]	GaAlAs	660	0.38	a) 15.8 b) 26.3 c) 39.5	35	—	a) 2.85 b) 4.75 c) 7.13
Junqueira et al. [37]	LED	660	—	26	50	—	3.3
Teichert et al. [38]	LED	664	—	—	400	—	11.45
Mima et al. [39]	LED	455	—	Denture: 37.5 Palate: 122	—	Denture: 24 Palate: 102	26 20
Mima et al. [40]	LED	455	—	37.5	—	24	26

InGaAlP: Indium-Gallium-Aluminum Phosphide LED: Light emitting diode GaAlAs: Gallium-aluminum-arsenide

Table 3. Characteristics of photosensitizers used in studies that fulfilled our eligibility criteria.

Authors et al.	Treatment of	Type of PS	PS Drug delivery	Pre-irradiation time (in minutes)	Concentration/s of PS used (in mg/dL)
Dovigo et al. [26]	Oral candidiasis	Curcumin	Topical	20	a) 0.74 b) 1.47 c) 2.95
Mima et al. [27]	Denture stomatitis	Porphyrin derivative	Denture surface were sprayed with the PS	30	50
Scwingel et al. [28]	Oral candidiasis	MB	Topical	1	45
Costa et al. [29]	Oral candidiasis	Erythrosine	Topical	1	4.5
Pupo et al. [30]	Oral *Candida* suspensions in 96 well plates	MB	—	5	10
		TBO			10
Costa et al. [31]	Oral *Candida* suspensions in 96 well plates	Erythrosine	—	5	12
Costa et al. [32]	Oral *Candida* suspensions in 96 well plates	Rose-Bengal	—	5	2.3
		Erythrosine			2.3
Martins Jda et al. [33]	Oral candidiasis	MB	Topical	1	10

Mang et al. [34]	Killing of cultured *Candida* species	Porphyrin derivative	—	60-1440	2.5
Mima et al. [35]	Experimental oral candidiasis	Porphyrin derivative	Topical	30	a) 40 b) 50 c) 100
Souza et al. [36]	Oral *Candida* suspensions in 96 well plates	a) MG b) MB c) TBO	—	5	a) 10 b) 10 c) 10
Junqueira et al. [37]	Experimental oral candidiasis	MB	Topical	5	10
Teichert et al. [38]	Experimental oral candidiasis	MB	Topical	10	a) 25 b) 27.5 c) 30 d) 35 e) 40 f) 45 g) 50
Mima et al. [39]	Denture stomatitis	Porphyrin Derivative	Denture and palate surfaces were sprayed with the PS	30	50
Mima et al. [40]	*Candida* growth on dentures	Porphyrin Derivative	Denture surfaces were sprayed with the PS	30	5

MB: Methylene Blue MG: malachite Green PS: Photosensitizer TBO: Toluidine Blue