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REVIEW

Strengthening collaborations at the Biology-Physics interface: trends in antimicrobial photodynamic therapy

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Abstract

The unbridled use of antimicrobial drugs over the last decades contributed to the global dissemination of drug-resistant pathogens and increasing rates of life-threatening infections for which limited therapeutic options are available. Currently, the search for safe, fast, and effective therapeutic strategies to combat infectious diseases is a worldwide demand. Antimicrobial photodynamic therapy (APDT) rises as a promising therapeutic approach against a wide range of pathogenic microorganisms. APDT combines light, a photosensitizing drug (PS), and oxygen to kill microorganisms by oxidative stress. Since the APDT field involves branches of biology and physics, the strengthening of interdisciplinary collaborations under the aegis of biophysics is welcome. Given this scenario, Brazil is one of the global leaders in the production of APDT science. In this review, we provide detailed reports of APDT studies published by the Laboratory of Optical Therapy (IPEN-CNEN), Group of Biomedical Nanotechnology (UFPE), and collaborators over the last 10 years. We present an integrated perspective of APDT from basic research to clinical practice and highlight its promising use, encouraging its adoption as an effective and safe technology to tackle important pathogens. We cover the use of methylene blue (MB) or Zn(II) porphyrins as PSs to kill bacteria, fungi, parasites, and pathogenic algae in laboratory assays. We describe the impact of MB-APDT in Dentistry and Veterinary Medicine to treat different infectious diseases. We also point out future directions combining APDT and nanotechnology. We hope this review motivates further APDT studies providing intuitive, vivid, and insightful information for the readers.

Keywords Antimicrobial resistance · Methylene blue · Photodynamic inactivation · Photosensitizer · Zn(II) porphyrin

Introduction

Light-based therapeutic approaches have been investigated in different areas of health science with promising results to treat a broad spectrum of diseases. The idea of using light as a therapeutic tool seems to be a recent and futuristic advance; however, it dates back to ancient times (more than 3000 years ago) when skin diseases were treated by bioactive compounds from plants that showed certain therapeutic effects when exposed to sunlight (Daniell and Hill 1991).

Phototherapy became a science at the beginning twentieth century, when Niels Finsen, a Danish medical scientist, published his first book, in which he described the treatment of skin diseases using monochromatic light (Finsen 1901). Subsequently, in 1903, he was awarded the Nobel Prize in Physiology or Medicine: "*in recognition of his contribution to the treatment of diseases, especially cutaneous*

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tuberculosis, with concentrated light radiation, whereby he has opened a new avenue for medical science".

In the same period, Oscar Raab, a German medical student under the supervision of Herman Von Tappeiner, accidentally observed that acridine orange (an exogenous photoactive compound) excited by light could inactivate stained protozoa. Later, Von Tappeiner, together with Jodlbauer, discovered that this interaction was oxygen-dependent, and named the term *photodynamische wirkung* (photodynamic effect) (Daniell and Hill 1991).

The photodynamic effect relies on the use of endogenous or exogenous photosensitizing compounds (PSs) and the application of a light source whose emission overlaps with the PS absorbance to kill cells, by inducing oxidative stress through the generation of reactive oxygen species (ROS). The photons emitted by the light source are absorbed by the PS molecule that goes to its first singlet excited state. Upon returning to its ground state, the PS can pass by its triplet state and react with biological targets *via* (i) charge transfer reactions (type I mechanism) forming $O_2^{\bullet-}$, H_2O_2, and $HO^{\bullet}$; and/or (ii) transfer of energy to oxygen, producing $^1O_2$ (type II mechanism) (Fig. 1) (Fong et al. 2017; Souza et al. 2021b).

In parallel to the scientific breakthroughs regarding the association between photochemical compounds and visible light made in the 1900s, there were meaningful therapeutic progresses in the field of infectious diseases with the emergence of sulfa and beta-lactam antimicrobial classes in the 1930s and 1940s, respectively. The following decades led to a scientific and pharmaceutical transformation, especially in the period between the 1950s and 1970s, which was called the Golden Era of the discovery of novel antibiotics classes. Consequently, the 1970s and 1980s were marked by scarce studies involving the photodynamic effect against microorganisms, especially because antibiotics remained highly effective against infectious diseases.

However, in the last decades, the massive use of antibiotics has contributed to the emergence, dissemination, and persistence of drug-resistant pathogens. Worryingly, infections that could be treated in the past became more challenging, and the selection and rapid spread of resistant populations of microorganisms emerged as a global phenomenon. Indeed, the World Health Organization (WHO) recognizes the urgent need for therapeutic alternatives since drug-resistant pathogens could kill about 10 million humans per year by 2050, becoming a major threat to public health worldwide.

As several microbial species can mutate and acquire resistance, big pharmaceutical companies are facing difficulties in developing novel antimicrobial classes, and few new antimicrobial compounds have been advancing in the clinical trial phases. As a consequence, antimicrobial photodynamic therapy (APDT), or photodynamic inactivation (PDI, usually used for in vitro studies), resurges as a promising approach to treat topical and/or local infections. Indeed, light technology in Health Sciences is a burgeoning area since the laser development by Theodore Maiman in 1960. Currently, dedicated light sources using lasers or light-emitting diodes (LEDs) allow offering an advance in the treatment of localized infectious diseases to assist in microbial drug-resistance emergence.

Herein, we report a review of the activities of the Laboratory of Optical Therapy (IPEN-CNEN), Group of Biomedical Nanotechnology (UFPE), and collaborators in the last 10 years, which aim to bring insights toward understanding the role played by APDT in the killing of different microorganisms (Fig. 2). We decided to use the abbreviation APDT, even for in vitro studies, throughout the manuscript to facilitate comprehension by the readers. Our studies are

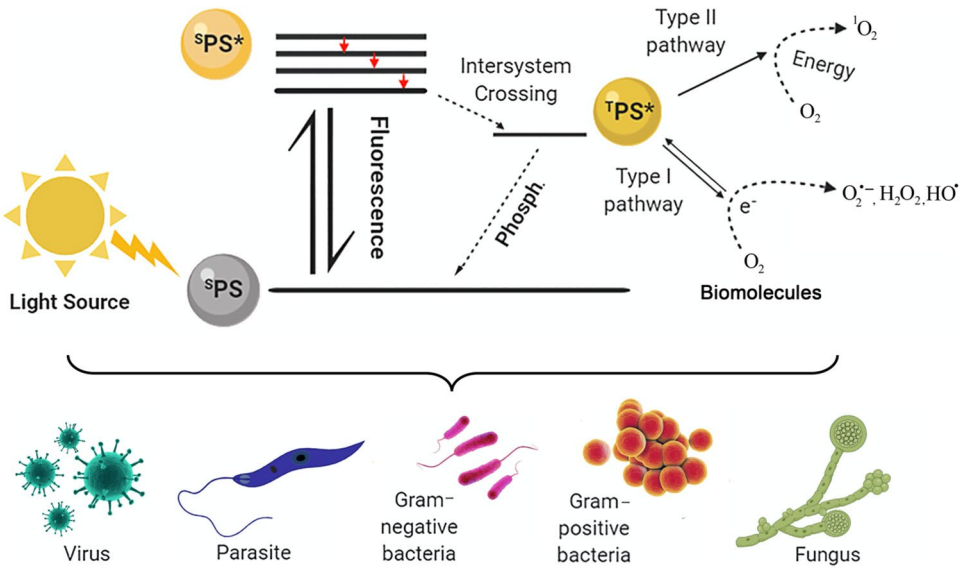

Fig. 1 Schematic representation of ROS production ($^1O_2$, $O_2^{\bullet-}$, H_2O_2, $HO^{\bullet}$) upon light absorption in APDT. PS photosensitizer, Phosph. phosphorescence. Adapted from Souza et al. (2021a), with permission from Elsevier

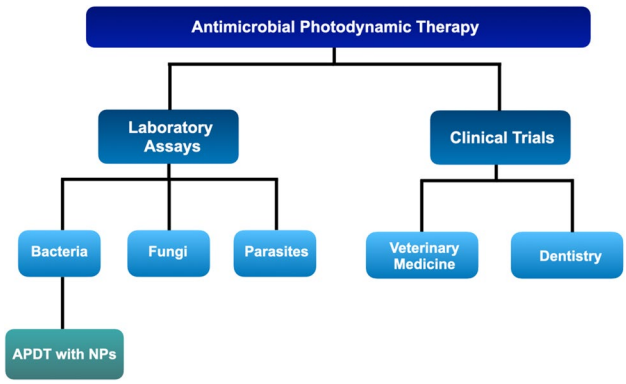

Fig. 2 The block diagram illustrates an overview of the topics addressed in this manuscript

focused on the use of methylene blue (MB) or Zn(II) porphyrins combined with red or blue light, respectively. We join the knowledge from Physical, Chemical, and Biological Sciences to present an integrated perspective from basic research to clinical trials. We also highlight future directions in this field.

APDT on pathogenic microorganisms in laboratory assays

Bacteria

Bacterial antimicrobial resistance represents a global threat to public health. The overuse of antibiotics has been considerably accelerating the development of resistant strains, making it difficult the treatment of numerous infections, thus resulting in high mortality rates. According to the Center for Disease Control and Prevention (CDC), multidrug-resistant *Pseudomonas aeruginosa*, vancomycin-resistant *Enterococci*, extended-spectrum beta-lactamase-producing *Enterobacteriaceae,* and methicillin-resistant *Staphylococcus aureus* (MRSA) are among the serious threat category of drug-resistant bacteria with great potential to spread and therefore become a challenge. In line with this, the WHO classified these as critical- or high-priority pathogens (Simpkin et al. 2017; Nadimpalli et al. 2018; Engel 2020).

In this regard, APDT has been evaluated against a wide range of bacteria. Table 1 summarizes the parameters used in the studies below reported, which are related to MB-mediated APDT (MB-APDT) in bacterial killing.

Sabino et al. tested the potential of MB-APDT against various drug-resistant bacteria. By using a red LED, several species were exposed to different radiant exposures ranging from 0 to 25 J/cm^2. As a result, it was observed a 4-log reduction against *Enterococcus faecium* (ATCC BA-2127 and vancomycin-resistant) at 5 J/cm^2. The killing rate was even better against three strains of *S. aureus* (ATCC 25923, MRSA, and vancomycin-resistant) and three strains of *Acinetobacter baumannii* (ATCC 19606 and carbapenem-resistant OXA-23- and OXA-143-positive) species. At a lower light dose of 4 J/cm^2, there was a 5-log decrease for both species regardless of the bacterial strains. In the same study, the light dose was increased to achieve the same killing response for *Klebsiella aerogenes* (ATCC 13048 and carbapenem-resistant NDM-1-positive) and *P. aeruginosa (*ATCC 27853 and carbapenem-resistant strains: GES-5, SPM-1, and VIM-2 producers). At 25 J/cm^2, all *K. aerogenes* strains were effectively inactivated, while all *P. aeruginosa* strains were killed at 20 J/cm^2, thus resulting in a 5-log decrease in both cases. Remarkably, in all experiments, either the wild-type or drug-resistant strains were equally susceptible to MB-APDT (Sabino et al. 2020). Additionally, *Escherichia coli* was reduced in 3-log using the same light parameters and MB concentration (Sabino et al. 2019). It is worth noting that in another work Sabino et al. proposed a mathematical model to calculate the lethal light doses for any microorganism, which allows a direct comparison among studies (Sabino et al. 2019).

It has been also demonstrated that MB-APDT was effective to kill different bacterial strains of *Klebsiella pneumoniae* producing medically important β-lactamase enzymes. These confer resistance of various Gram-negative bacteria to most β-lactams antibiotics. All strains were susceptible to MB-APDT. Moreover, there was a significant inhibition of β-lactamase enzymes in all strains at sublethal MB-APDT light doses (Anjos et al. 2020).

The success of APDT is thought to be dependent on the multi-target features of this technique, which can damage different biomolecules and cellular structures at the same time. Sabino et al. assessed the possible biochemical mechanisms of MB-APDT on carbapenem-resistant strains of *K. pneumoniae* using light parameters outlined in Table 1. The authors attributed protein degradation as the primary cause of bacterial killing following APDT (Sabino et al. 2023).

The impact of MB-APDT has also been reported with impressive results in oral pathogens. In Dentistry, microorganisms are organized in biofilms, which are biological communities where microbial cells are embedded in polymeric matrixes produced by them. *Actinobacillus actinomycetemcomitans*, a Gram-negative anaerobic bacillus, has been widely investigated as one of the major bacterial species present in periodontitis, a gum disease. In addition, *A. actinomycetemcomitans* has been associated with other important diseases, such as infective endocarditis, which can lead to significant cardiac consequences for those affected by this disease. By using a red laser and MB, Alvarenga and collaborators reported that *A. actinomycetemcomitans* biofilm was significantly reduced in 3-logs after 5 min. This outcome indicates that MB-APDT may offer a positive

Table 1 Parameters applied by the selected studies with bacteria

Species/strain	PS	PS concentration (μM)	PIT (min)	Light source	λ_{max} (nm)	Irradiance (mW/cm^2)	Light dose (J/cm^2)	Exposure time (s)	Outcome
E. faecium ATCC BA-2127 and vancomycin-resistant	MB	100	10	LED	660	100	5	50*	4-log reduction (Sabino et al. 2020)
S. aureus ATCC 25923, MRSA, VRSA	MB	100	10	LED	660	100	4	40*	5-log reduction (Sabino et al. 2020)
A. baumannii ATCC 19606, OXA-23, OXA-143	MB	100	10	LED	660	100	4	40*	5-log reduction (Sabino et al. 2020)
K. aerogenes ATCC 13048 and NDM-1	MB	100	10	LED	660	100	25	250*	4-log reduction (Sabino et al. 2020)
P. aeruginosa ATCC 27853, GES-5, SPM-1,VIM-2	MB	100	10	LED	660	100	20	200*	5-log reduction (Sabino et al. 2020)
E. coli	MB	100	10	LED	660	100	25	250*	3-log reduction (Sabino et al. 2019)
K. pneumoniae ATCC 700603, KPBr-1, ICB-KPS3.2	MB	100	10	LED	660	100	16	160	3-log reduction (Anjos et al. 2020)
K. pneumoniae ATCC 700603, KPBr-1, and ATCC BAA1705	MB	100	10	LED	660	100	20	200*	~3-log reduction (Sabino et al. 2023)
A. actinomycetemcomitans	MB	100	1	laser	660	250*	75	300	3-log reduction (Alvarenga et al. 2015).
S. aureus, S. agalactiae, S. dysgalactiae, and *C. bovis*	MB	50	5	LED	662	100	0.5-18	5-180	Complete killing (Sellera et al. 2016b)

MRSA methicillin-resistant *S. aureus*, *VRSA* vancomycin-resistant *S. aureus*. *PS* photosensitizer. *PIT* pre-irradiation time (also named period of PS dark incubation), λ_{max} maximum emission of the light source

*Calculated by the authors

impact and help to overcome some of the challenges faced by patients with periodontitis (Alvarenga et al. 2015).

Envisaging the use of APDT to treat mastitis in dairy cows, the potential of MB-APDT was investigated against antibiotic-resistant bacterial strains isolated from bovine mastitis (*S. aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Corynebacterium bovis*) and the pathogenic algae *Prototheca zopfii*. Although light dose for complete inactivation varied according to the microorganism, MB-APDT showed to be a promising approach for preventing antibiotic residues in milk (Sellera et al. 2016b).

Fungi

Fungal infections account for over 1.5 million deaths per year worldwide affecting particularly those with immunocompromised conditions that are at risk of developing serious and life-threatening forms of the disease. Because of the growing concern about several fungal diseases, the WHO released in late 2022 the first fungal priority list. *Candida albicans, Cryptococcus neoformans, Candida auris*, and *Aspergillus fumigatus* are the species that belong to the critical group of fungal pathogens. They can cause invasive fungal infections, thus being potentially lethal to most people that present chronic diseases, such as HIV, cancer, and diabetes mellitus. Additionally, these opportunistic pathogens are also likely to develop drug resistance (Cowen 2008; Revie et al. 2018).

In this regard, APDT has been broadly employed against several fungal species. Table 2 summarizes the parameters related to APDT in the fungal killing applied in the studies described herein. It has been demonstrated that sublethal light doses of MB-APDT reduced not only the germ tube formation in yeast of *C. albicans* but also increased susceptibility to oxidative stress, and damaged the cell wall. As a result, MB-APDT reduced the capacity of *C. albicans* to cause a systemic infection in mice. Besides, the minimal inhibitory concentration (MIC) of the antifungal drug fluconazole was reduced following MB-APDT, which suggests the combination

Table 2 Parameters applied by the selected studies with fungi

Species/strain	PS	PS concentration (μM)	PIT (min)	Light source	λ_{max} (nm)	Irradiance (mW/cm^2)	Light dose (J/cm^2)	Exposure time (s)	Outcome
C. albicans (yeats and biofilm)	ZnPHex	0.15–1.5	10	LED	410	24.1	4.3	180	Complete eradication of yeasts at 0.8 and 1.5 μM 89% decrease in biofilm at 0.8 μM (Souza et al. 2022)
C. albicans	ZnPEt	10	10	LED	460	150	81*	540	3-log reduction (Viana et al. 2015)
C. albicans	MB	50	10	Laser	660	75	9-27 (sublethal)	120-360	Decreased germ tubes formation Increased ROS susceptibility Reduced MIC of fluconazole Extended animal survival in a systemic infection (Kato et al. 2013)
C. albicans (azole-resistant)	MB	100	10	LED	660	100	25	250*	3-log reduction (Sabino et al. 2020)
C. neoformans KN99a, CAP59, 208820 and 208819 strains	MB	10	30	Lamp (Lumacare®)	665	40	60	1500*	~ 0.5-log reduction against *KN99a* and *208820* 2-log reduction against *CAP59* and *208819* (Prates et al. 2013)
C. neoformans (azole-resistant)	MB	100	10	LED	660	100	25	250*	3-log reduction (Sabino et al. 2020)

PS photosensitizer, *PIT* pre-irradiation time, λ_{max} maximum emission of the light source

*Calculated by the authors

of therapies is a useful approach to treating *C. albicans* infections (Kato et al. 2013). Posteriorly, we demonstrated that an azole-resistant *C. albicans* strain was equally susceptible to MB-APDT as the non-resistant strain (Sabino et al. 2020).

The impact of MB-APDT against *C. neoformans* was also investigated. This species contains a polysaccharide capsule that is thought to be a dominant virulence factor of this fungus. Moreover, it confers protection against the host defense mechanisms, interfering with host immune responses. Prates et al. showed a 0.5-log reduction in the serotype A KN99α of *C. neoformans* strain (containing the capsule over the entire cell) following MB-APDT. However, the killing effect was more pronounced when the isogenic *cap59* mutant strain (with a defective capsule) was treated, resulting in nearly a 2-log reduction at the same experimental conditions. The authors also tested the potential of MB-APDT against two other strains, a laccase positive (2008820) and a laccase negative strain (208219). Laccase is an enzyme present in the *C. neoformans* cell wall that is also strongly involved with the virulence of this species. Cells were exposed to MB-APDT resulting in a dose-response curve similar to that observed for KN99α and *cap59* mutant strains, achieving 0.5 and approximately 2-log reduction in laccase positive and negative strains, respectively (Prates et al. 2013). More recently, Sabino et al. exposed *C. neoformans* azole-resistant strains to MB-APDT. Both strains were susceptible to APDT regardless of the light dose, resulting in a 3-log reduction at

the highest light dose compared to untreated yeasts cells (Sabino et al. 2020) (Table 2).

Although MB is a well-established PS to mediate APDT, issues such as aggregation and photobleaching motivate the search for new photoactive compounds. Using the tetracationic Zn(II) *meso*-tetrakis(*N*-ethylpyridinium-2-yl)porphyrin (ZnTE-2-PyP^{4+}; ZnPEt) and blue light, Viana et al. evaluated the ability of ZnPEt-APDT individually or combined with CdTe quantum dots (QDs) stabilized with mercaptosuccinic acid to kill *C. albicans* yeasts (Table 2). ZnPEt-mediated APDT showed higher fungicidal activity than when combined with QDs. The authors assumed that the ZnPEt conjugation with QDs prevents the PS cellular uptake, hence reducing fungal inactivation (Viana et al. 2015).

Later, Souza et al. used the more amphiphilic Zn(II) *meso*-tetrakis(*N*-n-hexylpyridinium-2-yl)porphyrin (ZnTnHex-2-PyP^{4+}; ZnPHex) to mediate the photoinactivation of yeasts and biofilms of *C. albicans* strains under blue LED irradiation. ZnPHex-mediated APDT (ZnPHex-APDT) at concentrations of 0.8 and 1.5 µM promoted the eradication of *C. albicans* yeasts ATCC 10231 and ATCC 90028, respectively. Moreover, ZnPHex-APDT led to an 89% decrease in biofilm viability at the lowest PS concentration, causing structural changes, disorganization with reduced hyphae tangles, and more substrate exposure. No considerable cytotoxicity was observed in mammalian cells (Souza et al. 2022).

Parasites

Some parasitic diseases, such as schistosomiasis, leishmaniasis, and Chagas disease, are considered neglected tropical diseases since they are often ignored by global funding agencies and have very limited resources. The lack of investments in novel therapeutic strategies is reflected in the very low number of new commercially available drugs in the last decades. For cutaneous leishmaniasis (CL), for example, another point that deserves to be highlighted is the limited use of standard antileishmanials. The long treatment regimen, toxicity, route of administration, and selection of resistant phenotypes are some of the reasons that limit their use (Madusanka et al. 2022). In this scenario, APDT has also attracted attention as a promising and innovative technology for the treatment of CL. Table 3 summarizes the parameters related to APDT in *Leishmania* applied in the in vitro studies presented in this review.

Leishmaniasis is caused by protozoan parasites of the genus *Leishmania*, and it is transmitted to humans and animals by the bite of female sandflies. More than 20 *Leishmania* species cause CL, including *L. amazonensis* and *L. braziliensis*, which are the most common species in Brazil. *Leishmania* parasites are dimorphic organisms, and our studies have focused on both *Leishmania* forms: promastigotes, which develop within sandflies, and amastigotes, which live and multiply within macrophages in the host.

To verify the best parameters for eradicating *L. amazonensis* promastigotes, Aureliano et al. used MB-APDT varying the PS concentration, pre-irradiation time, and red LED

Table 3 Parameters applied by the selected studies with parasites

Species/strain	PS	Concentration (µM)	PIT (min)	Light source	λ_{max} (nm)	Irradiance (mW/cm^2)	Light dose (J/cm^2)	Exposure time (s)	Parasite killing
L. braziliensis	ZnPEt	10	10	LED	455	300	90	600	90% promastigotes ~ 40% amastigotes (Andrade et al. 2018)
L. amazonensis and *L. braziliensis*	ZnPHex	1.25	5	LED	410	19.1	3.4	180	> 99% to both *Leishmania* species of promastigotes ~ 64% of *L. amazonensis* amastigotes (Souza et al. 2021a)
L. major and *L. braziliensis*	MB	6.25	120	OLEDs	671	6.5	50	7560	51% *L. major* 87% *L. braziliensis* (Cabral et al. 2021a)
L. amazonensis	MB	50	10	LED	645	~ 350	106.2	300	80% (Aureliano et al. 2018)
Bioluminescent *L. amazonensis*	MB	100	10	LED	660	100	50	500	90% (Cabral et al. 2019)

PS: photosensitizer. PIT: pre-irradiation time. λ_{max}: maximum emission of the light source

irradiation parameters. Furthermore, the authors analyzed the possible death pathways after MB-APDT. The authors reported that longer exposure times promoted higher parasite killing. The PS uptake by parasites was similar regardless of the pre-irradiation time and MB concentration. Following MB-APDT, the authors observed alterations in cell membrane permeability and mitochondrial depolarization. Additionally, ultrastructural alterations of the parasite cells were reported, such as intense vacuolation of the cytoplasm, increase in the mitochondria-kinetoplast complex, cell shrinkage, and small blebs in the flagella and cell membrane of the parasite. According to the authors, these findings indicate that apoptosis is involved in MB-APDT of *L. amazonensis* (Aureliano et al. 2018).

Later, Cabral and coworkers also used MB-APDT to evaluate its action against a bioluminescent strain of *L. amazonensis* monitoring the parasite burden by bioluminescence. MB-APDT inactivated promastigote forms of *L. amazonensis* in a light dose-dependent manner. In vivo, the authors induced CL in mice by inoculating bioluminescent *L. amazonensis* in the paw. Mice were divided into two treatment groups (1 and 2 sessions) and monitored for 4 weeks. Both MB-APDT groups (100 µM and 150 J/cm^2) exhibited a significant parasite burden reduction compared to the control group. However, 2 sessions of APDT sustained a lower parasite load than 1 session. Additionally, 2 sessions were clinically better, especially considering the inflammatory process associated with CL (Cabral et al. 2019).

More recently, Cabral et al. explored the potential of organic LEDs (OLEDs) for APDT against *L. major* and *L. amazonensis*. OLEDs were compared to LEDs by using three different phenothiazine dyes: MB, new methylene blue (NMB), and 1,9-dimethyl-methylene blue (DMMB). The results showed that both OLEDs and LEDs are effective light sources for APDT regardless of the PS. The death of *Leishmania* spp. occurred even at very low irradiance, demonstrating the promising use of OLEDs as wearable light bandages for ambulatory care of CL (Cabral et al. 2021a).

Using the cationic and hydrophilic ZnPEt combined with blue light, Andrade et al. reported parasite damage in promastigotes forms of about 90% and a reduction of around 40% in the number of intracellular amastigotes of *L. braziliensis* on infected macrophages following ZnPEt-APDT (Andrade et al. 2018). On the other hand, Souza et al. used ZnPHex-APDT and reached a cell death > 99% of the promastigote forms of *L. braziliensis* and *L. amazonensis* (Souza et al. 2021a). Besides, the authors reported a reduction of 64% in the number of amastigotes of *L. amazonensis* per macrophage at a very low concentration of ZnPHex. In both studies, the authors monitored the mitochondrial membrane potential of the parasites (ΔΨm). They observed an evident hyperpolarization of ΔΨm following ZnPEt-APDT, while ZNPHex-APDT revealed an intense ΔΨm depolarization. Both alterations in ΔΨm led to the death of the parasites. The authors associated such different behavior with the higher lipophilicity of ZNPHex, which facilitates its interaction with cell membranes promoting a greater intracellular PS bioavailability. In both studies, no noteworthy toxicity was observed against mammalian cells (Andrade et al. 2018; Souza et al. 2021a).

Clinical studies of APDT in Veterinary Medicine: a new field of possibilities

Veterinary Medicine has significantly progressed over the last decades. Advances in medicine and science led to the improvement of diagnostic technologies and the advent of new therapeutic approaches that can benefit animals. In Brazil, MB-mediated APDT (MB-APDT) has triggered interest in different areas, and studies have been performed to treat infectious diseases and difficult-to-treat wounds in companion, wild, and food-producing animals.

Small animal practice is undoubtedly the most promising area for APDT in Veterinary Medicine. Human-pet bonds have significantly increased in recent years, and in many countries, pet owners regard their pets as family members. Consequently, the search for prolonging the longevity of companion animals led to the adoption of modern therapeutic approaches. Additionally, similarly to human medicine, the emergence of drug-resistant pathogens is a phenomenon that currently threatens companion animals, making the development of effective and safe novel broad-spectrum non-antibiotic approaches desirable.

In companion animals, the use of MB-APDT has been successfully reported to treat dermatological diseases. Table 4 summarizes the APDT protocols used in Veterinary Medicine studies presented in this review. A case report demonstrated that a single APDT session effectively treated unilateral otitis externa caused by a high-risk carbapenem-resistant *Pseudomonas aeruginosa* (Sellera et al. 2019). Samples collected immediately and after 7 and 14 days following MB-APDT were negative for *P. aeruginosa*, whereas the complete resolution of clinical signs was achieved after 7 days. Additionally, the susceptibility of this *P. aeruginosa* strain to MB-APDT was evaluated. Results revealed that this resistant strain was equally susceptible to the treatment when compared to a drug-sensitive *P. aeruginosa* strain.

Another case report highlighted the use of MB-APDT for canine dermatophytosis caused by *Microsporum canis* (Cabral et al. 2021b). MB-APDT was performed over the lesions in two sessions with an interval of 7 days until complete cure. A complete clinical cure was achieved after 21 days, without reports of recurrence after a 6-month follow-up period. More recently, the association between MB-APDT and oral administration of itraconazole was

Table 4 Protocols applied by the selected clinical studies in Veterinary Medicine

Animal	Infectious disease	PS	Concentration	PIT (min)	Light source	λ_{max} (nm)	Power (mW)	Energy (J)	Exposure time/point (s)	Sessions	Outcome
Dog	Otitis	MB	100 µM	5	Laser	660	100	8	80 (6 points)	1	Clinical cure (Sellera et al. 2019)
Dog	Dermatophytosis	MB	500 µM	10	Laser	650	40	6	150 (4 points)	2× with 7-day intervals	Clinical cure and no recurrence during the 6-month follow-up (Cabral et al. 2021b)
Cat	Sporotrichosis	MB	500 µM	10	Laser	660	100	9	90 (6 points)	5 (3× a week in the first month + 2× every 2 weeks)	Clinical cure and no recurrence during the 3-month follow-up (Cabral et al. 2022)
Sheep	Lymphadenitis	MB	60 µM	5	Laser	660	100	4	40 (1 point)	1×/week until wound closure	Lesions successfully regressed and no recurrence during the 6-month follow-up (Sellera et al. 2016a)
Cows	Digital dermatitis	MB	0.01%	5	LED	660	2100	84	40 (1 point)	2× with 14-day intervals	Clinical cure, a significant increase in type I and III collagen levels, and absence of spirochetes (Sellera et al. 2021)
Bulls	Surgical wound	MB	0.01%	5	Laser	660	100	2	200 (30 points)	2×/week until 28 days	Better post-surgical profile and animals were faster discharged (Valandro et al. 2021)
Sheep	Abscess	MB	0.01%	5	Laser	660	100	18	180 (5 points)	1	Clinical cure (Sellera et al. 2015)
Cow	Sole ulcer	MB	0.1%	10	Laser	660	100	4	40*	1×/week until wound closure	Clinical cure (Sellera et al. 2018)
Penguin	Pododermatitis	MB	300 µM	5	Laser	660	100	4	40 (5 points)	1×/week until wound closure	Lesions successfully regressed and no recurrence during the 6-month follow-up (Sellera et al. 2014)
Penguins	Pododermatitis	MB	300 µM	5	Laser	660	100	4	40*	3×/week until wound closure	Faster healing and shorter treatment time than the antibiotic group (Nascimento et al. 2015)
Snakes	Stomatitis	MB	0.01%	5	Laser	660	100	8	80 (1 point)	1×/week for 3 months	Reduction of inflammatory signs and caseous material, and *K. pneumoniae* lost resistance phenotype for seven antibiotics (Grego et al. 2017)

PS photosensitizer, *PIT* pre-irradiation time, λ_{max} maximum emission of the light source

*The number of points varied according to the lesion size

also demonstrated in a cat with sporotrichosis (Cabral et al. 2022). The animal received low doses (around 35 mg/day) of itraconazole daily for 50 days. Five sessions of MB-APDT were also performed in 45 days. Following 2 weeks after the end of treatment, the cat was clinically cured, and the fungal culture was negative for *Sporothrix* spp. Neither side effects nor recurrences were observed after a 3-month follow-up.

Currently, the massive use of antibiotics in food-producing animals has contributed to the risk of contamination of milk, meat, and other animal-derived food products by antibiotic residues or antibiotic-resistant bacteria. Critically, the increasing rates of antibiotic-resistant bacteria in these animals have raised serious public health concerns worldwide since the transmission of these pathogens to humans may occur. In light of this, the feasibility of MB-APDT to manage caseous lymphadenitis abscesses in sheep after surgical drainage was investigated (Sellera et al. 2016a). Ten animals had their abscesses drained and were exclusively treated by MB-PDT once a week until complete recovery. The average time of healing was 15.3 days, and no adverse effects or recurrences were observed during 6 months of follow-up.

More recently, MB-APDT was compared with oxytetracycline (gold standard) in the treatment of bovine digital dermatitis, an infectious disease associated with *Treponema* species that causes lameness in cattle worldwide (Sellera et al. 2021). Clinical and histological findings revealed that MB-APDT promoted better results than oxytetracycline for the elimination of spirochetes, production of collagen, and wound healing. In another study, MB-APDT was combined with chlorhexidine and zinc oxide ointment in the wound healing process after rumenostomy, a surgical procedure (Valandro et al. 2021). A control group with only chlorhexidine and zinc oxide ointment was used for clinical comparative analysis. Results demonstrated that MB-APDT promoted faster wound closure, fewer post-surgery complications, and more animals were discharged from the cattle care facility during the same period. Other successful applications of MB-APDT included the treatment of cutaneous streptococcal abscesses in sheep (Sellera et al. 2015) and a case of sole ulcer in a cow (Sellera et al. 2018).

Exotic, zoo, and wildlife medicine, not unlike other areas, has evolved over the last decades. The clinical practice for these animals is complex and challenging due to the wide range of species (i.e., mammals, birds, reptiles, amphibians, and fishes), physiology, behavior, and species-specific diseases. Diseases of microbiological origin are among the main concerns, making APDT a potential ally for the treatment and preventive care of infectious diseases on this front.

The first study described the use of MB-APDT to treat penguins suffering from pododermatitis, one of the most frequent and challenging diseases that affect seabirds in captivity or rehabilitation centers (Sellera et al. 2014). A subsequent study with pododermatitis in penguins, compared MB-APDT versus classical antibiotic treatment, revealing that MB-APDT promoted better results than antibiotics alone without inducing adverse side effects (Nascimento et al. 2015).

Snakes in captivity develop infectious stomatitis caused by Gram-negative bacteria, which could impair the production of antivenom. In collaboration with the Butantan Institute, the use of MB-APDT in snakes was exploited (Grego et al. 2017). All snakes presented clinical improvement, determined by the reduction of inflammatory signs and the absence of caseous material. More interestingly, the microbiological analysis revealed that *K. pneumoniae* isolates before and after MB-APDT were single clones with 100% of genetic similarity that lost resistance phenotype for seven antibiotics of four distinct classes. This finding indicates that MB-APDT induces alterations in DNA and could be a new strategy to promote the loss of plasmid in bacteria.

Clinical studies of APDT in Dentistry: a worldwide benchmark

The clinical use of APDT has emerged in Dentistry with promising results for the inactivation of a broad diversity of microorganisms and in the treatment of several oral infectious diseases. A large number of studies have been performed using different APDT protocols, including light sources and irradiation parameters, and different types of photosensitizers. Particularly in Brazil, MB-APDT has been the most used in clinical studies due to its effectiveness, low cost, and safety. Additionally, MB up to 1% does not require registration by the National Health Surveillance Agency (ANVISA), which simplifies its adoption by mainstream Brazilian dentists. Table 5 summarizes the APDT protocols in Dentistry applied in the studies presented in this review.

Periodontitis is a disease of the tissue surrounding the tooth structure and could be a challenge in dental practice. This infection may result in exacerbated inflammatory response that can lead to alveolar bone reabsorption. Due to its antimicrobial properties, APDT has been investigated as an adjuvant approach for periodontitis. A randomized controlled clinical trial using 30 patients with chronic periodontitis was performed to evaluate the best parameters for APDT in the periodontal pocket (Alvarenga et al. 2019). Microbiological analyses were performed to quantify microorganisms before and after treatments. MB was used in an aqueous solution or a surfactant vehicle (0.25% sodium dodecyl sulfate solution) and then irradiated with a red laser. No antimicrobial effects were achieved with aqueous MB-APDT. In contrast, MB in the surfactant vehicle produced a microbial reduction in the group irradiated for 5 min. This might be explained by the reduction of MB aggregation when it was exposed to the surfactant.

Table 5 Protocols applied by the selected clinical studies in Dentistry

Disease	PS	Concentration	PIT (min)	Light source	λ (nm)	Power (mW)	Energy (J)	Exposure time (s)	Sessions	Outcome
Candida-associated denture stomatitis	MB	450 µg/mL	10	Laser	660	100	28	280/cm^{2}*	8 (2×/week for 4 weeks)	Reduced inflammatory signs after 15 days A similar fungal burden to the miconazole group (de Senna et al. 2018)
Oral candidiasis	MB	450 µg/mL	1	Laser	660	30	3	10/point (9 points)	1	Complete eradication of *Candida* spp. Improved clinical condition (Scwingel et al. 2012)
Chronic periodontitis	MB	100 µM	1	Laser	660	100	30	300/point (1 point)	1	Microbial reduction due to disaggregation of MB (Alvarenga et al. 2019)

PS photosensitizer, *PIT* pre-irradiation time
*The area varied for each patient

On the other hand, candidiasis is the most common fungal disease in Dentistry. *Candida* spp. exist as commensals in the oral cavity of most individuals, but can also act as opportunistic pathogens, especially in immunocompromised patients. Moreover, *Candida*-associated denture stomatitis is a frequent fungal infection that affects individuals with a denture. In this regard, a comparative study between MB-APDT and oral administration of miconazole gel 2% was performed on the oral mucosa and prosthesis of 36 patients with denture stomatitis (de Senna et al. 2018). Clinical evaluation was based on the degree of oral mucosa erythema, whereas microbiological analysis was performed to assess the reduction of *Candida* spp. in both palatal mucosa and prosthesis. The results showed that MB-APDT was significantly more effective than miconazole in attenuating the inflammatory process after 15 days. Additionally, MB-APDT and miconazole groups presented similar fungal burdens after treatment.

Another study was proposed to treat oral candidiasis in HIV-infected patients (Scwingel et al. 2012). For this purpose, twenty-one patients were divided into three groups (oral administration of fluconazole; low-level laser therapy; and MB-APDT). Patients were clinically evaluated, and microbiological analysis was performed before, immediately after, and 7, 15, and 30 days after the treatments. Although fluconazole was effective, it did not prevent the recurrence of candidiasis. Low-level laser therapy did not promote clinical improvement nor lead to a reduction in *Candida* spp. In contrast, MB-APDT inactivated 100% of *Candida* spp., and no recurrences were observed in the patients up to 30 days after the treatment.

APDT assisted by nanoparticles

Recent developments involving nanotechnology have allowed exploring the use of different nanosystems to assist APDT. Indeed, light can induce a phenomenon called localized surface plasmon resonance (LSPR), which is a collective oscillation of electrons from the conduction band on the surface of metallic nanoparticles (NPs). In this case, NPs can be used to enhance APDT by improving PS excitation and ROS generation (Ribeiro et al. 2018). To take advantage of LSPR, it is required to have (i) spectral overlap between the NP extinction band and the PS absorption band and (ii) PS and NP be at a distance smaller than 10 nm.

LSPR was first tested using riboflavin (Rb, 40 µg/mL) together with spherical AgNPs coated by pectin in APDT of *E. coli* (Gram-negative) and *Streptococcus mutans* (Gram-positive) (Ribeiro et al. 2018). Using a blue LED (455 nm, 90 mW/cm^2), Ribeiro et al. achieved a 3-log reduction of *S. mutans* after 6 min of irradiation when bacteria were incubated with the Rb-AgNP system for 10 min. Rb-mediated

APDT alone reduced less than 1-log of *S. mutans*. However, no significant difference was found in the performance of Rb alone or combined with AgNPs for *E. coli*. The authors attributed the differences in APDT of both bacteria to the low PS uptake by *E. coli.*

Later, Farooq et al. explored MB-APDT and negatively charged Au nanoshells on *S. aureus* (Farooq et al. 2019). The bacteria were incubated with MB alone (4 µM) or combined with NPs for 10 min and irradiated by a red LED (659 nm, 47 mW/cm^2). Complete eradication was reached after 1 min of irradiation using the MB-AuNP system. On the opposite, MB alone was able to eradicate *S. aureus* only after 3 min of irradiation.

More recently, Ag nanoprisms coated with poly-4(styrenesulfonate) anionic chains were associated with MB (45 µM) in APDT of resistant *S. aureus*, isolated from bubaline mastitis (Rodrigues et al. 2021). Using a red LED (660 nm, 45.8 mW/cm^2) after a PIT of 10 min, the authors showed that the MB-AgNP system was able to eradicate bacteria following 6 min of irradiation. In contrast, MB-APDT reduced only about 1-log after 9 min of irradiation. The authors also demonstrated increased ROS production for MB-AgNP systems.

NPs can be also employed as a vehicle to improve the PS delivery in the target. Envisaging the use of APDT for internal infections, Toledo et al. reported the use of superparamagnetic iron oxide nanoparticles (SPIONs) as a nanocarrier of MB since these NPs can be directed to specific sites using an external magnetic field (Toledo et al. 2020). SPIONs were covered with a double silica layer leading to the hybrid material magnetite-silica-MB and tested in APDT of *E. coli* using a red LED (665 nm, 16.2 mW/cm^2). The authors showed that the release time of MB from SPIONs influences bacteria inactivation but promotes a dose-response curve depending on exposure time.

Final remarks

As microbial infections have been imposing challenges for humanity, the demand for the development of technologies to strengthen the global response in the combat of antimicrobial-resistant microorganisms has been increasingly necessary. The results reported here demonstrate that APDT mediated by MB or Zn(II) porphyrins is effective against a wide range of microorganisms of clinical interest, including bacteria, fungi, protozoa, and pathogenic algae. Besides, APDT could decrease virulence factors, reduce the in vivo pathogenicity, inhibit important enzymes linked to resistance, and broaden the susceptibility to antibiotics. Clinical studies in Dentistry and Veterinary Medicine demonstrate the potential of this technique.

An interesting aspect is that APDT is not only a border technology in respect of multidisciplinary professional profiles involved, but also has been improving through two-way exchanges between research and clinical. In this context, future directions tend to have an increasing participation of nanotechnology and the combination of APDT with lower doses of conventional antimicrobials to potentiate its outcomes. Additionally, APDT could be enhanced by the use of inorganic salts (e.g., potassium iodide), surfactants (e.g., sodium dodecyl sulfate), aggregation destabilizing compounds (e.g., urea), solubility enhancers (e.g., cyclodextrin), delivery systems (e.g., hydrogels), and well-controlled clinical studies are very welcome.

In summary, this review shows that the collaborative work of life science professionals and physicists may significantly contribute to the consolidation of APDT as a promising weapon to fight infectious diseases. We hope this review opens an avenue to guide future studies and be a useful reference for researchers and professionals that already work or intend to work in this area.

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Declarations

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed in the studies here reported. Besides, all procedures performed in studies involving human participants were following the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Consent to participate Not applicable.

Consent for publication Elsevier granted permission to reproduce Fig. 1 (license number 5518491193627).

Conflict of interest The authors declare no competing interests.

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