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Organic Light-Emitting Diodes as an Innovative Approach for Treating Cutaneous Leishmaniasis

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Antimicrobial photodynamic therapy (APDT) has been studied as a noninvasive therapy for treating cutaneous leishmaniasis to overcome challenges with current treatment, such as toxicity, resistance, and need for in-patient hospital treatment. Organic light-emitting diodes (OLEDs) have emerged as an attractive technology that can provide wearable light-emitting materials that are conformable to human skin. This makes OLEDs ideal candidates for APDT by light-bandages for ambulatory care. In this work, suitable OLEDs are successfully developed to match the absorbance of three photosensitizers: methylene blue, new methylene blue, and 1,9-dimethyl-methylene blue to inactivate two *Leishmania* species in vitro: *Leishmania major* and *Leishmania amazonensis*. Parasites are treated either by LED (20 mW cm^{-2}) or OLED (6.5 mW cm^{-2}) at increasing photosensitizer concentrations at a radiant exposure of 50 J cm^{-2}. 1,9-Dimethyl-methylene blue is the most potent photosensitizer, killing both strains at nanomolar concentrations. The effect of different intensities from the OLEDs (0.7, 1.5, and 6.5 mW cm^{-2}) are also explored and it is shown that effective killing of *Leishmania* occurs even at a very low intensity. These findings demonstrate the great potential of OLEDs as a new approach for ambulatory treatment of cutaneous leishmaniasis by APDT.

1. Introduction

Leishmaniasis is an important neglected tropical disease that affects over 200 countries across the world.[1] It comes in two forms—visceral (VL) and cutaneous leishmaniasis (CL). The illness is caused by the protozoan parasites of the genus *Leishmania* and transmitted by the bite of hematophagous female infected sandflies. It is closely related to poverty and lack of resources in many regions of low-income countries from Latin America, Asia, and Africa.[1,2]

According to the World Health Organization, CL is a problem in around 100 countries.[1] CL develops deep disfiguring and destructive ulcerated lesions that are difficult to treat and prone to secondary bacterial and fungal infections.[3] There are several limitations of current treatments. Pentavalent antimonials are the first-line drug of choice and they are based on a regimen of daily injections for a long period.[4] Alternatively, amphotericin B has been used for unresponsive cases and is administered by intravenous infusion.[4,5] Miltefosine, an anticancer drug (the only available in oral formulation) has also been applied for the treatment of CL. However, due to strains resistant to this compound, it has not been approved in many countries.

All of these treatments are associated with high toxicity, high cost, systemic long-term administration, and drug resistance.[4] These limitations lead not only to severe adverse and side effects but also to poor adherence to treatment and consequently relapses in the disease.[5] In addition, patients are dependent upon multiple daily injections, which are painful, making hospital treatment necessary.[4,5]

Antimicrobial photodynamic therapy (APDT) has emerged as a potential alternative treatment for CL with many advantages over the drugs currently used.[6] APDT involves the activation of a photosensitizer with a light source at a suitable wavelength in the presence of molecular oxygen in order to produce reactive oxygen species (ROS).[6] APDT is an attractive therapy because of its low cost, broad-spectrum (able to kill bacteria, fungi, viruses, and parasites), very low toxicity for mammalian cells, and being unlikely to produce resistance due to its mechanism of action. In addition, topical administration and immediate results may shorten the duration of treatment.[7]

Although there are many benefits of APDT, the widespread use of the therapy is limited by the need for specialized, bulky, and expensive light sources. Lasers are coherent light sources that have been widely used for a long time for small areas, mostly in the field of dentistry, or as an antitumor therapy for oral or superficial skin lesions.[8] However, their high-cost, and

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(in many cases) high level of technical support required, turns them into an undesirable tool to be used in mainstream medicine.[9,10] Furthermore, they emit narrow beams which present a hazard to the eye and are ill-adapted to uniform illumination of an area to be treated.

To overcome these issues, incoherent inorganic light-emitting diodes (LEDs) have been implemented as alternative light sources with broader emission spectra, able to deliver light over a large area, being suitable for the treatment of larger skin lesions.[11,12] Despite the high efficiency of LEDs, they intrinsically are point sources so manufacturing them might still be a challenge in terms of emission uniformity, besides light being unable to be delivered evenly onto curved surfaces.[13] At present, inorganic LEDs for APDT are bulky machines found in a limited number of hospitals and clinics.

Advances in organic light-emitting materials now provide an alternative route to light sources for medical applications. Organic light-emitting diodes (OLEDs) have many attractive features for medical applications[14–17] such as being lightweight, thin light sources that are flexible and inexpensive. These properties make them ideal for wearable light sources for ambulatory APDT.[18] They have recently been reported as novel light sources for bacteria inactivation.[14] A particular advantage of OLEDs is that they are intrinsically area light sources, and so well matched to treating topical infections and lesions, which require uniform illumination over their surface area. OLEDs have been shown to be very effective for treating skin cancer in human patients.[18] Although OLEDs have been described as promising light sources for antitumor therapy, so far their antimicrobial efficiency has only been reported for treating bacterial infections.[14] Thus, it is worth investigating their potential as light sources for CL.

In this paper, we evaluated the effectiveness of OLEDs combined with three different phenothiazine dyes: methylene blue (MB), new methylene blue (NMB), and 1,9-dimethyl-methylene blue (DMMB), against two species of *Leishmania* promastigotes (*Leishmania major* and *Leishmania amazonensis*), which cause CL.[19] Phenothiazine-based photosensitizers have been chosen not only due to their ability to generate ROS but also because they are cost-effective and so could be widely used.[19,20] Our study started by designing and making suitable OLEDs, and then applying them to in vitro assays in 96-well plates. Our results demonstrate that OLEDs are effective for all three photosensitizers, suggesting the great potential of OLEDs to be used as light sources for ambulatory APDT to treat cutaneous leishmaniasis.

2. Experimental Section

2.1. OLED Fabrication and Characterization

OLEDs were fabricated by thermal evaporation at a base pressure of 3×10^{-7} mbar (EvoVac, Angstrom Engineering Inc.). Materials used in the OLED fabrication were as follows: 300 nm aluminum as anode, 50 nm 2,2′,7,7′-tetrakis(*N*,*N*′-di-*p*-methylphenylamino)-9,9′- spirobifluorene (Spiro-TTB) doped with 2,2′-(perfluoronaphthalene-2,6-diylidene)dimalononitrile (F6-TCNNQ) (4 wt%) as hole-transport layer, 10 nm *N*,*N*′-di(naphtalene-1-yl)-*N*,*N*′-diphenylbenzidine (NPB) as electron-blocking layer, 40 nm NPB doped with [Ir(MDQ)2(acac)] (10 wt%) as emission layer, 10 nm bis-(2-methyl-8-chinolinolato)-(4-phenyl-phenolato)-aluminium(III) (BAlq) as hole-blocking layer, 70 nm 4,7-diphenyl-1,10-phenanthroline (BPhen) doped with cesium as electron-transport layer, 20 nm silver as semi-transparent cathode, and 80 nm NPB as capping layer. The OLEDs were encapsulated in the glove box under a nitrogen atmosphere with glass lids and UV-curable epoxy glue (Norland NOA68).

The electrical characteristics of OLEDs were measured with a source-measure unit (Keithley 2400, Keithley). The EL spectra of the OLEDs were obtained using a spectrograph (MS125, Oriel) coupled to a charge-coupled device (CCD) camera (DV420-BU, Andor). The irradiance of OLEDs was measured with an optometer (P9710, Gigahertz Optik). The emission uniformity of OLEDs was measured on the center of each well in the 96-well plate with a fiber-coupled CCD camera. Then, the irradiances of each position were calculated according to the relative light intensity measured by a CCD camera.

2.2. Parasites

L. amazonensis (MHOM/BR/73/M2269) and *L. major* (MHOM/IL/80 Fredlin) promastigotes were grown at 28 °C in M199 medium (Sigma-Aldrich) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco Invitrogen Corporation), HEPES (40×10^{-3} M) pH 7.4 (Sigma-Aldrich), hemin (2.5 mg mL^{-1}) (Sigma-Aldrich), and adenosine (10×10^{-3} M) (Sigma-Aldrich).[21]

2.3. Phenothiazine-Based APDT of *Leishmania* spp

The activity of APDT against both *Leishmania* species was carried out by the addition of serial dilutions of MB (100 µL, 0×10^{-6} to 100×10^{-6} M) (Sigma-Aldrich), NMB (0×10^{-6} to 10×10^{-6} M) (Sigma-Aldrich), or DMMB (0×10^{-9} to 3000×10^{-9} M) (Sigma-Aldrich) into a 96-well plate. Parasites were then seeded at 1×10^6 per well in a final volume of 200 µL and incubated with the corresponding photosensitizer (MB, NMB, or DMMB) for 10 min to allow the photosensitizer uptake by the parasites before irradiation. Afterwards, APDT was performed using two different light sources (LED or OLED) in separated plates. Light parameters were set as described in **Table 1**.

Parasite toxicity of the three photosensitizers with no light was assessed by their incubation at increasing concentrations of MB (0×10^{-6} to 100×10^{-6} M), NMB (0×10^{-6} to 10×10^{-6} M), or DMMB (0×10^{-9} to 3000×10^{-9} M), in the dark for 2 h.

Table 1. Light source parameters for APDT.

	LED	OLED
Radiant exposure [J cm^{-2}]	50	50
Intensity [mW cm^{-2}]	20	6.5
Time [min]	41′ 39″	128′ 12″
λ [nm]	660 ± 12.5	671 ± 140

Control groups receiving only light (LED or OLED) were also evaluated using the same parameters described in Table 1.

Then, parasite viability assay was assessed at the end of each experiment by the addition of resazurin (Alamar blue, Sigma-Aldrich), a nonfluorescent compound that undergoes a cellular metabolic reduction becoming highly fluorescent in the presence of living cells. Briefly, a stock solution (10 µL,1.1 mg mL^{-1}), dissolved in PBS, was added in each well and incubated for 5 h at 28 °C.[22] Afterwards, the fluorescence intensity was determined by using a plate reader (Gen5 Reader, BioTek) at λ_{exc} = 530 nm and λ_{em} = 590 nm. Results were then normalized and expressed as a percentage of live parasites.

2.4. OLED-APDT Light Dose Study of *L. amazonensis*

OLED-APDT efficiency at various radiant exposures was evaluated against *L. amazonensis* in the presence of increasing concentrations of DMMB, as this was the most potent photosensitizer. Parasites were seeded in 96-well plates as described previously and exposed to light of 6.5 mW cm^{-2} at different radiant exposures as shown in **Table 2**.

We also explored reduced OLED intensity of 1.5 mW cm^{-2} and compared with LED at 8 J cm^{-2} (20 mW cm^{-2}). To further investigate OLED-APDT at lower intensity, it was reduced to 0.7 mW cm^{-2} at the lowest radiant exposure delivering 2 J cm^{-2}. Experiments were performed as described in **Tables 3** and **4**. Then, parasite viability assay was assessed at the end of each experiment as mentioned in "Phenothiazine-based APDT of *Leishmania* spp." section.

2.5. Statistical Analysis

Data were obtained in triplicates and were analyzed by two-way analysis of variance (ANOVA). Differences were considered statistically significant when $p < 0.05$.

3. APDT Light Sources for Treating Cutaneous Leishmaniasis

3.1. Requirements of Light Sources for APDT

The light source is one of the three key components in APDT, and advances in light-emitting materials provide new opportunities that we explore in this paper. The light source is so important because it excites the photosensitizers to the excited state, leading to the generation of ROS. The first requirement for the light source is therefore that it can efficiently excite the photosensitizer. This means that it must emit light at a wavelength (or range of wavelengths) that is strongly absorbed by the photosensitizer. A good match of the emission spectrum of the light source to the absorption of the photosensitizer can reduce the light output needed to achieve a given level of ROS production. In addition to having a suitable emission spectrum, the light sources also need to be efficient. Highly efficient light sources can lower the power consumption so that the light sources are able to reach high output intensity (radiant exitance) with low heat generation. For ambulatory APDT where the light sources are placed on human skin, reducing heat generation is particularly important as otherwise the light source could (in the worst case) burn the patient, or be very uncomfortable. The third requirement of the light source is that it should have high output intensity, in order to generate ROS fast enough for effective APDT. For large area treatment, the light source needs to have high uniformity in order to deliver similar light doses over an area. Last, but not least, APDT is developing toward ambulatory treatment, in which the patient can move around. So, the light sources will need to be lightweight and preferably flexible to adapt to the curvature of the human body.

Table 2. OLED-APDT exposure times to give different radiant exposures at intensity 6.5 mW cm^{-2}.

Time	Radiant exposure [J cm^{-2}]
5′ 7″	2
10′ 15″	4
20′ 30″	8
32′	12.5
64′ 6″	25
128′ 12″	50

Table 3. LED and OLED-APDT parameters at radiant exposure of 8 J cm^{-2}.

	LED	OLED	OLED
Radiant exposure [J cm^{-2}]	8	8	8
Intensity [mW cm^{-2}]	20	6.5	1.5
Time	6′ 39″	20′ 30″	88′ 52″

3.2. Development of OLEDs for APDT

Here we developed large area OLEDs, specifically for APDT on 96-well plates. A highly efficient red phosphorescent material bis(2-methyldibenzo [*f*,*h*]quinoxaline)(acetylacetonate) iridium(III) [$Ir(MDQ)_2(acac)$] was selected as the emitter for the OLEDs. In order to have an effective excitation of the photosensitizers, the OLEDs were designed in a top emitting geometry with a thick Al bottom electrode and a semitransparent silver top electrode (**Figure 1**a). The metal electrodes also act as reflecting mirrors so that the OLED is a planar microcavity. Therefore, by simply changing the layer thicknesses between the two metal contacts, the emission peak of OLEDs can be tuned. The electrical and optical performance of the OLEDs are shown in Figure 1b. A low turn-on voltage was achieved by using doped transport layers. Low driving voltage due to the doped transport layers is important because it reduces joule-heating and so enables higher light outputs to be reached. Another advantage of using doped layers is that the emission peak can be tuned by varying the transport layer thickness without changing the conductivity of the OLEDs. As a result, our large area OLEDs achieved a high intensity of 11 mW cm^{-2} at a current density of 45 mA cm^{-2} and applied voltage 3.96 V. The OLED emission

Table 4. OLED-APDT parameters at radiant exposure of 2 J cm^{-2}.

	OLED		
Radiant exposure [J cm^{-2}]	2	2	2
Intensity [mW cm^{-2}]	6.5	1.5	0.7
Time	5′ 7″	22′ 14″	47′ 36″

spectrum was tuned to peak at 671 nm, which matches the absorption bands of MB, DMMB, and NMB (**Figure 2**).

As explained above, uniformity of illumination is desirable, but it can be hard to achieve in large area OLEDs because of the high sheet resistance of the thin metal layer. The voltage-drop in the contact can induce a difference in the device driving voltage across the emitting area and affect the uniformity of emission. Here, we introduced a grid of silver contacts 2 µm thick to spread current around the edge of the emitting region of the top contact. We also made the bottom contact of aluminum 300 nm thick to minimize resistive losses. These measures led to light output being uniform within ±13% of its mean value at an irradiance of 6.5 mW cm^{-2} over the 31.9 mm by 40.9 mm emitting area (Figure S1, Supporting Information). This was considered a satisfactory level of uniformity for the APDT experiments.

OLEDs were prepared for APDT experiments on 96-well plates. The devices consisted of four OLED pixels of the design described above fabricated together on a single glass substrate. The size of each OLED pixel was 32 mm by 41 mm and it could fully cover 12 wells (Figure 1b) with uniform illumination. Four switches that can individually control each pixel were implemented so that light and dark conditions can be applied to different regions of the same well plate, as desired. This OLED geometry in combination with the 96-well plates enables many APDT experiments to be done at once, and the uniformity within a single well is better than that for LED illumination, as OLEDs generate light over an area rather than being point light sources.

3.3. Development of Inorganic LEDs for APDT

For comparison with the OLEDs, a light source consisting of inorganic LEDs was also made. It consisted of 12 LEDs (660 nm OSLON SSL, OSRAM) arranged in a 3 × 4 grid. LEDs emitting at a wavelength 660 nm were selected to match the absorption of the photosensitizers used in our experiments (Figure 2). The experimental setup also accommodates 96-well plates (Figure S2, Supporting Information) and illuminates simultaneously 12 wells from underneath, with distance 4 mm from the bottom of the well. It gives good beam uniformity around ±5% within one well. A constant current power supply for driving the LEDs was built with three user-selectable currents, which produced intensity range 12.5, 25, and 50 mW cm^{-2}.

4. Results

APDT is well known for giving results quickly due to the high amounts of ROS produced that are able to kill neighboring microbial cells. In order to measure the potential of three different phenothiazine dyes activated by either LED or OLED, the metabolic activity of parasites was measured directly after therapy. Light sources were set to deliver 50 J cm^{-2}: ≈42 min at 20 mW cm^{-2} for the LED and 128 min at 6.5 mW cm^{-2} for the OLED (see Table 1). The results are plotted in **Figure 3** and show that even at the lowest concentration, APDT produced a significant cellular inactivation of both *Leishmania* species. This inactivation was achieved by both light sources. In contrast, no inactivation was observed in most of the control experiments of i) light without photosensitizer, and ii) photosensitizer without light. However, for DMMB at 3000 × 10^{-9} M the photosensitizer without light did lead to some inactivation – 43.5 ± 2.8% for *L. amazonensis* and 27.3 ± 6.1% for *L. major* (Figure S3, Supporting Information).

Our results show that both OLEDs and LEDs are effective light sources for APDT, and work well with a range of

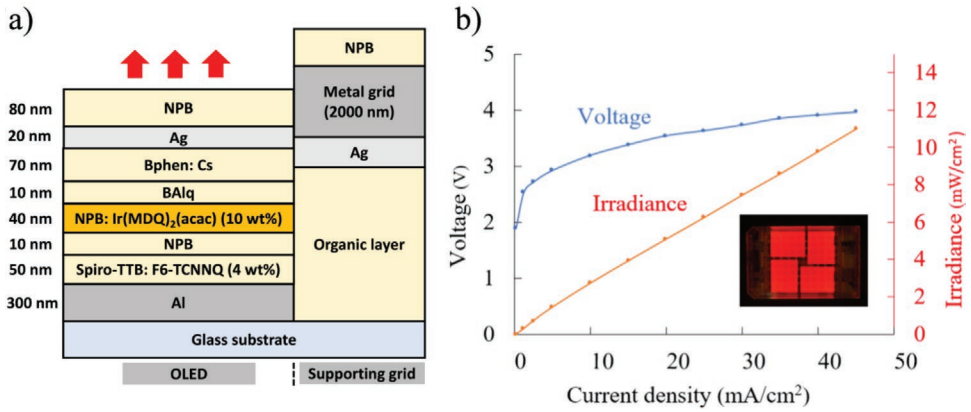

Figure 1. a) OLED structure designed for APDT and supporting grid structure for uniformity enhancement. b) Voltage–current density–irradiance (*V*–*J*–Irradiance) of OLED designed for APDT. The inset shows a photograph of four OLED pixels illuminating a 96-well plate.

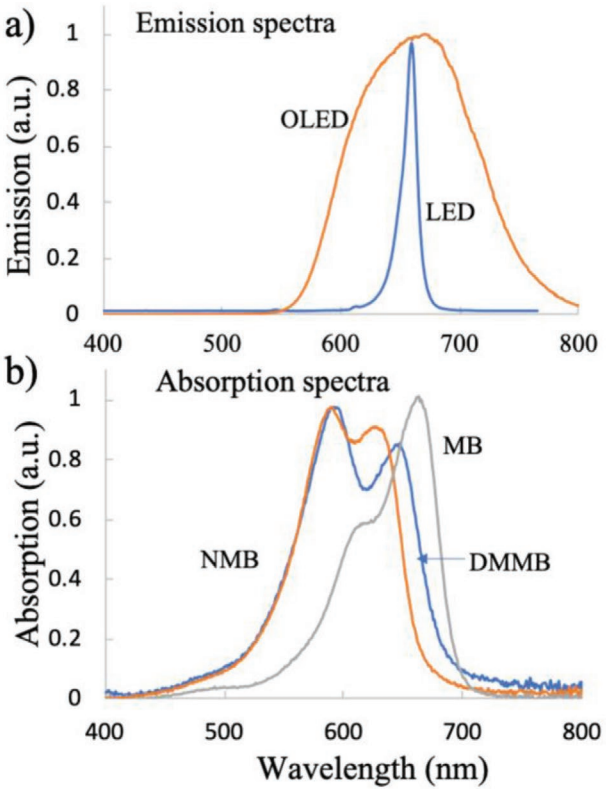

Figure 2. a) Electroluminescence spectra of OLED and LED. b) Absorption spectra of methylene blue (MB), new methylene blue (NMB), and 1,9-dimethyl-methylene blue (DMMB) in deionized water.

photosensitizers and/or *Leishmania* species. In some cases, the inactivation was larger for OLED-APDT (**Figure 4**). For example, for MB at 6.25×10^{-6} M, LED-APDT killed an average of 46.6 ± 1.6% and 53.3 ± 5.4% for *L. major* and *L. amazonensis*, respectively, whereas OLED-APDT, caused an inactivation of 51.2 ± 1.4% and 86.9 ± 0.6% for the same species. It is worth noticing that in terms of LED-APDT, both species showed a significant dependence on MB concentration. A similar dependence was observed for OLEDs with *L. major*. However, this trend was no longer observed when *L. amazonensis* was exposed to OLEDs, and a similar killing rate was observed across the concentration range from 6.25×10^{-6} to 100×10^{-6} M (Figures 3a,d and 4a,d).

The results for the photosensitizer NMB are shown in Figure 3b,e, and the same pattern is observed. Here it is very clear that OLEDs have a greater effect than LEDs on both *Leishmania* species, especially at lower concentrations (Figure 4b,e). An average of 90.5 ± 1.5% and 59.7 ± 3.8% of *L. amazonensis* and *L. major* were killed by OLED-APDT at 0.6×10^{-6} M, which is much more than for LED-APDT, which killed 58 ± 1.5% of *L. amazonensis* and 13 ± 3.1% of *L. major*.

When DMMB was used as the photosensitizer, LED-APDT and OLED-APDT provided similar results for the lowest and the highest concentrations (Figures 3c,f and 4c,f). An average of 37.3 ± 12.4% and 81.8 ± 4.2% of *L. major* were inactivated by the LED source at 187×10^{-9} and 3000×10^{-9} M, respectively. The killing rate of *L. major* achieved by the OLED was 42 ± 4.5% at 187×10^{-9} M and 82.5 ± 0.4% at 3000×10^{-9} M. For *L. amazonensis* the OLED killed 86.4 ± 0.9% and 92.9% ± 0.4% at 187×10^{-9} and 3000×10^{-9} M, respectively, whereas 70.4 ± 4.5% and 94 ± 0.4% were killed at the same concentrations by LEDs.

Indeed, *L. amazonensis* appears to be more susceptible to APDT than *L. major* under all conditions, i.e., regardless of the photosensitizer or light source. Overall, DMMB turned out to be the most potent photosensitizer since it is able to promote an effective killing rate at low (nanomolar) concentrations.

As *L. amazonensis* was the most susceptible cell line to APDT, parasites were exposed to increasing concentrations of DMMB (the best photosensitizer), under a wide range of radiant exposures (from 2 to 50 J cm^{-2}), in order to further investigate the effectiveness of OLED APDT. **Figure 5**a shows the photosensitizer concentration–response curve fit at radiant exposures from 2 to 50 J cm^{-2}, obtained by using 6.5 mW cm^{-2} for times from 5 to 128 min (Table 2). The results show that at 8 J cm^{-2}, the rate of killing is not significantly different from that for 50 J cm^{-2}. The parasites are likely being inactivated in the first 20 min: 85.4 ± 4.5% for 187×10^{-9} M photosensitizer and 98.4 ± 0.4% for 3000×10^{-9} M, photosensitizer.

The intensity supplied by the OLED was then reduced in order to deliver 8 J cm^{-2} at 1.5 mW cm^{-2} over 90 min. The LED intensity was kept at 20 mW cm^{-2}, delivering the same radiant exposure in 6 min, as described in Table 3. As shown in Figure 5b, APDT was more effective for the OLED at the lowest concentration (187×10^{-9} M), promoting significant parasite reduction when treating *L. amazonensis* cells with DMMB. The OLED achieved 86.5 ± 4.9% inactivation for 6.5 mW cm^{-2} illumination, and 72.4 ± 6.5% inactivation for 1.5 mW cm^{-2} illumination, whereas the LED achieved 61.4 ± 3.5% inactivation at 20 mW cm^{-2}. A similar trend of OLEDs operating at low intensities being more effective than the LED source is also seen at the other concentrations.

Moreover, according to Figure 5a, even at the lowest radiant exposure (2 J cm^{-2}), parasite load was reduced significantly, for all the DMMB concentrations. Data show that 64.7 ± 3.9% and 94.1 ± 0.4% of cells were killed when treated with DMMB at the lowest and highest concentration. Therefore, we used these data as the basis for the next step in optimizing OLED-APDT and lowered OLED intensity even further to 0.7 mW cm^{-2}. The OLED was set to deliver 2 J cm^{-2} over ≈50 min to compare OLED-APDT at different intensities, as shown in Table 3. The results are shown in Figure 5c, and the death rate is unchanged despite the reduced intensity. For example, 96.5 ± 0.9% of cells were inactivated at a photosensitizer concentration of 1500×10^{-9} M, suggesting the pronounced impact of OLED as a potential tool to be used for antileishmanial therapy.

5. Discussion

In this work, we have successfully developed OLED light sources for APDT of cutaneous leishmaniasis. We then evaluated their effectiveness in vitro using three phenothiazine-based dyes as photosensitizers. We find that both OLEDs and LEDs are able to inactivate both *L. major* and *L. amazonensis* regardless of the photosensitizer concentration.

MB is a well-known dye that has been widely employed as a photosensitizer due to its absorption lying in the red region

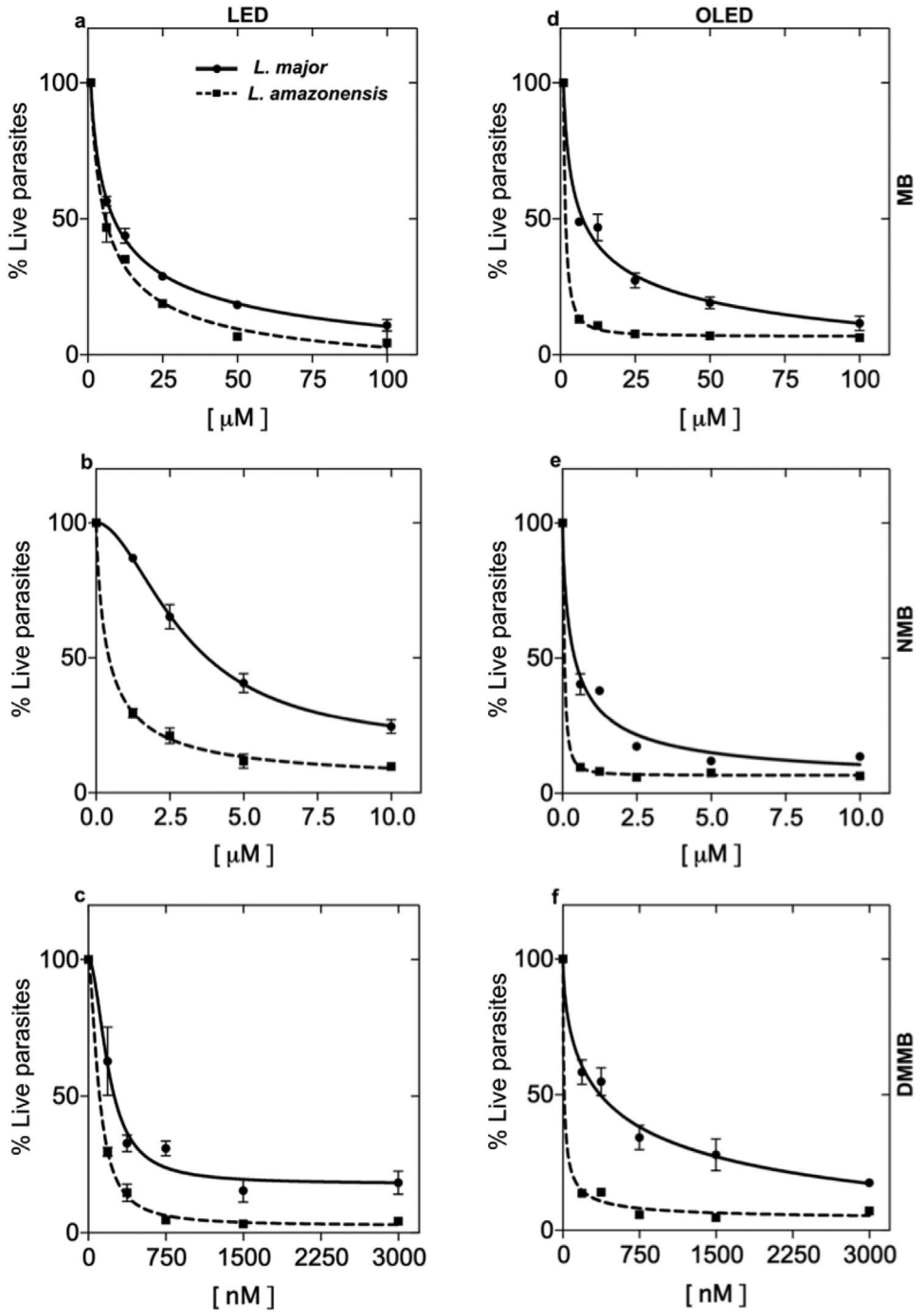

Figure 3. *L. major* and *L. amazonensis* promastigotes treated with LED and OLED-APDT at 50 J cm^{-2} in the presence of increasing concentrations of a,d) MB, b,e) NMB, and c,f) DMMB. Values shown represent the mean ± SD.

of the spectrum, with two absorption peaks at 609 and 660 nm (Figure 2). MB is able to generate high amounts of ROS either by Type I reaction, such as O_2^-, OH^-, and H_2O_2, or by Type II reaction, achieving a singlet oxygen quantum yield of nearly 0.44.[20] In terms of application, it has been demonstrated to promote promising results against a wide range of microbial species, mostly bacteria, and fungi, including resistant strains.[23,24] As an antileishmanial agent, in vitro and in vivo studies show its potential, reporting not only its ability to reduce the parasite burden in preclinical trials but also to treat human patients with impressive cosmetic results.[25–27]

However, the vast majority of APDT light sources are LED-based and although they allow illumination over a large area, occasionally light is unable to be delivered uniformly as a result of shadow formation. We have shown that OLEDs provide a new light source for PDT of *Leishmania* species that is at least

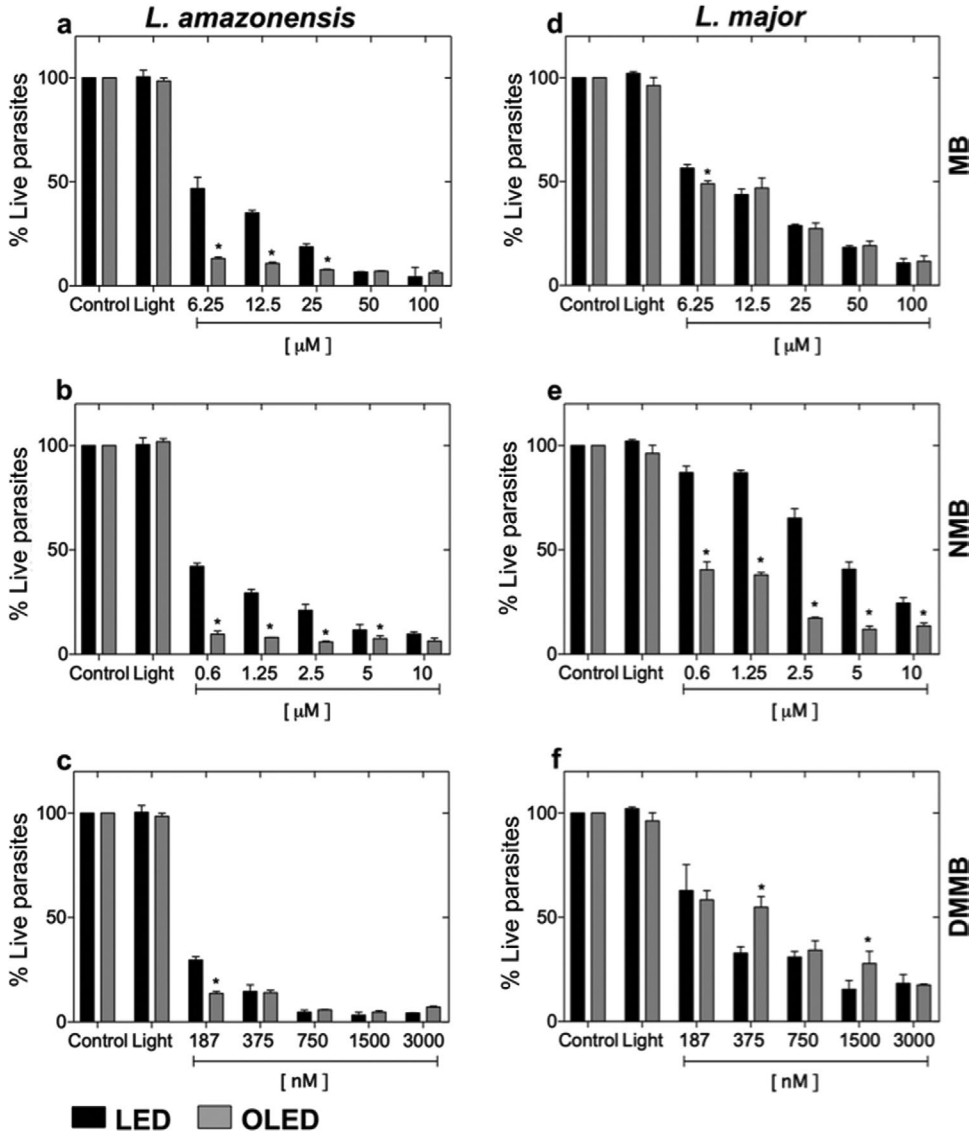

Figure 4. Comparison between OLED and LED-APDT. *L. major* and *L. amazonensis* promastigotes treated at 50 J cm^{-2} in the presence of increasing concentrations of a,d) MB, b,e) NMB, and c,f) DMMB. Values shown represent the mean ± SD. Statistically significant differences observed between OLED and LED are marked with $^{*}p < 0.05$.

as effective as LEDs and offers further advantages. OLEDs are more compact, can be flexible, and give uniform light emission over an area. Although the lower OLED irradiance resulted in a longer irradiation time to achieve the same light doses used for LED-APDT, this is not a problem as the OLEDs could potentially be worn as a light source and longer exposure time gives more time for oxygen to diffuse to the region to be treated. Another feature is that OLEDs have a broader emission spectrum than LEDs, which gives good overlap with the absorption of all the photosensitizers (Figure 2). The wider emission spectrum also means that OLEDs could excite both monomers and dimers of MB simultaneously. This would promote both Type I and Type II reactions, and so may enhance parasite inactivation.[28,29]

Phenothiazinium derivatives are cationic molecules that are able to interact more efficiently with the negatively charged parasitic membranes, rather than anionic or neutral compounds, turning them into attractive agents for APDT.[30] A considerable number of MB analogues, such as NMB and DMMB, have arisen as new alternatives to MB because of their higher lipophilicity, as well as their ability to generate 35% and 21% more singlet oxygen than MB.[19,20] As a result, NMB has been shown to promote antimicrobial effects against different microorganisms such as *Streptococcus mutans* and *Candida albicans* biofilms, including animal models.[31,32]

In our work, we have demonstrated the great potential of NMB under red light illumination, in which we have been able to efficiently inactivate the parasites at concentrations nearly 100 times lower than those of MB. This significant effect was also even clearer for OLED-APDT. As the NMB maximum absorption peaks is at 590 nm, with another peak at 630 nm, NMB only partially absorbs LED irradiation because the LED emission consists of a narrow peak at 660 nm. In contrast the

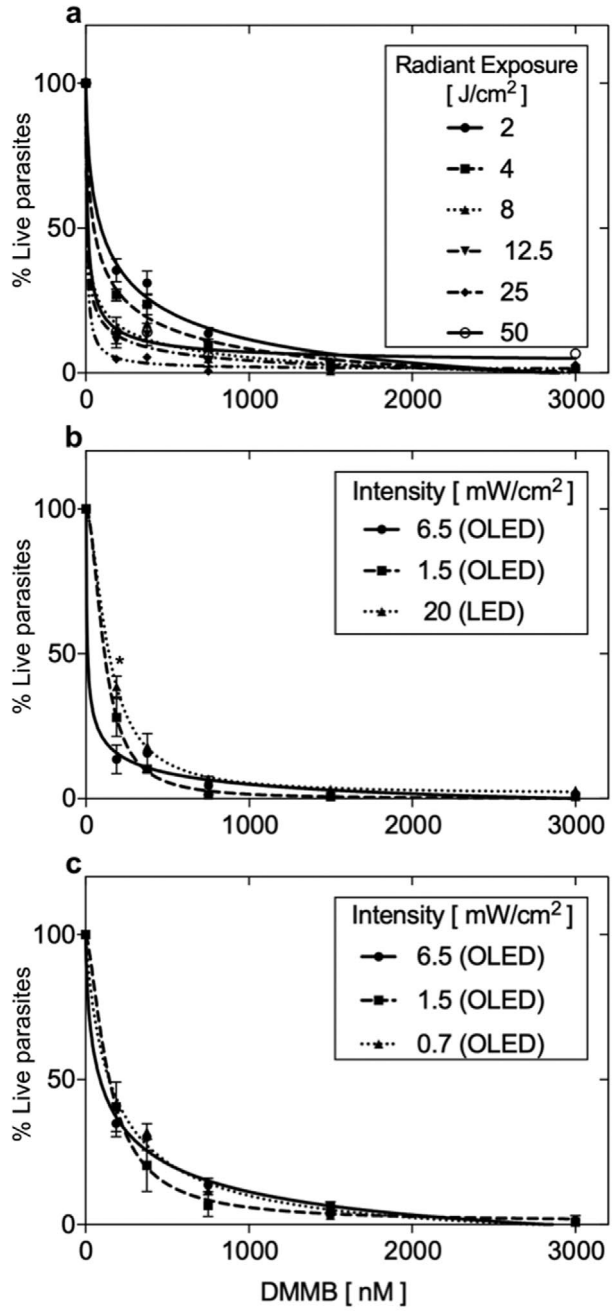

Figure 5. *L. amazonensis* promastigotes treated with LED and OLED-APDT in the presence of increasing concentrations of DMMB. a) OLED at different radiant exposures and intensity of 6.5 mW cm^{-2}. b) OLED at 8 J cm^{-2} and intensities of 6.5 and 1.5 mW cm^{-2}. LED-APDT was performed at the same radiant exposure and intensity of 20 mW cm^{-2}; c) OLED-APDT at 2 J cm^{-2} and different intensities (0.7, 1.5 and 6.5 mW cm^{-2}). Values shown represent the mean ± SD (* denotes statistically significant differences between LED and OLED-PDT).

wider emission spectrum of the OLED can excite the NMB, which provided an increased death rate.

DMMB is a dimethylated derivative of MB that has been shown to be more resistant to photobleaching.[33] In addition, it is more effective as can be seen from the excellent improvement in the killing rate of *Leishmania* species at low (nanomolar) concentrations (Figures 3e,f and 4e,f). As mentioned above, the improvement is due to its increased lipophilicity (positive log *P* value), in contrast to the hydrophilic nature of MB (log $P < 0$).[33] Thus, DMMB as a cationic lipophilic compound is most likely to target and accumulate in the *Leishmania* mitochondrion.[33] Since *Leishmania* parasites possess only one large ramified mitochondrion, we suggest that APDT disrupts the membrane potential resulting in a pronounced cellular killing.

There have been very few reports to date regarding the phototoxic effects of DMMB, and most of them are related to its use as an antibacterial agent under LED-based light sources.[34,35] As far as we know, our work is the first report of the use of DMMB against *Leishmania* species. We find that both light sources significantly decrease the parasite's survival under DMMB application in a photosensitizer concentration-dependent manner.

To further investigate the potential of OLEDs, we explored lower intensities at the same radiant exposure (2 J cm^{-2}), resulting in effective killing even at low photosensitizer concentrations. We found very effective killing by 20 min of illumination at 1.5 mW cm^{-2}. Illumination for longer times using 0.7 mW cm^{-2} was slightly less effective (Figure 5c). This may be because 2 J cm^{-2} at mW cm^{-2} is insufficient to fully overcome the antioxidant capacity of *L. amazonensis*. We also note that 20 min of illumination delivering 8 J cm^{-2} at 6.5 mW cm^{-2} gave very effective inactivation compared with other intensities, especially at low DMMB concentrations (Figure 5a,b). Taken together, these findings suggest that the use of an appropriate light dose in combination with a suitable intensity is needed to achieve the largest antileishmanial effect.

The effectiveness of APDT depends on many variables including the parameters of the illumination, the properties of the photosensitizer, and how it interacts with the cells, the light source, and oxygen. *Leishmania* spp. contain superoxide dismutase, peroxidases, and a series of thiol-containing proteins that act as antioxidants for ROS generated by Type I reactions.[36,37] In addition, there are differences in the biology of *Leishmania* species, including in the redox system.[38] Our observation that *L. amazonensis* is killed more effectively than *L. major* can be explained by the latter containing peroxidases capable of scavenging high levels of H_2O_2, making it more tolerant of ROS.[39,40] As a result, a Type II reaction is preferred to target *L. major* since there are not endogenous antioxidant defenses for singlet oxygen. This may also explain why DMMB was particularly effective. It has a high singlet oxygen quantum yield (by Type II reaction), so more oxidative stress was generated in the cells in the short-term, achieving faster killing at lower concentrations.[33]

In conclusion, we have demonstrated that OLEDs are very promising light sources for APDT. Importantly, we have shown that they are very effective for APDT to kill two different strains of *Leishmania* parasites. Additionally, a recent study by Pereira et al. showed no cytotoxicity of the three photosensitizers on mammalian cells.[41] We also found that this could be achieved at relatively low intensities and very low photosensitizer concentration. Our in vitro results suggest that OLED-APDT is a promising direction for potential ambulatory care of patients who suffer from cutaneous leishmaniasis that should be followed up by in vivo studies.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

I.D.W.S. is a founder and shareholder of Ambicare Health Ltd, which develops wearable light sources for the treatment of acne.

Data Availability Statement

The data that support the findings of this study are openly available from the University of St Andrews research portal at https://doi.org/10.17630/554cbd41-80aa-4c22-8dca-95798ee346eb, ID/Reference number: 273471450.

Keywords

antimicrobial photodynamic therapy, flexible light sources, *Leishmania* parasites, low irradiance photodynamic therapy, methylene blue, organic light-emitting diode, phenothiazine photosensitizer

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