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Temperature increase inside LED-based illuminators for *in vitro* aPDT photodamage studies

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ABSTRACT

Antimicrobial PhotoDynamic Therapy (aPDT) is an emerging strategy aimed at the eradication of bacterial infections, with a special focus on antibiotic-resistant bacteria. This method is easy to apply, not expensive and particularly interesting in case of bacteria that spontaneously produce the required photosensitizers. In the framework of a project aimed at the development of an ingestible pill for the application of aPDT to gastric infections by *Helicobacter pylori*, a LED-based illuminating prototype (LED-BIP) was purposely designed in order to evaluate the photodamage induced by light of different wavelengths on porphyrin-producing bacteria. This short paper reports about temperature tests performed to assess the maximum exposure time and light dose that can be administered to bacterial cultures inside LED-BIP without reaching temperatures exceeding the physiological range.

Introduction

Conventional pharmacological therapies are becoming less effective towards several bacterial infections due to the increasing antibiotic resistance developed by pathogenic bacteria [1,2]. In this context, antimicrobial PhotoDynamic Therapy (aPDT) is a promising alternative since it relies on different mechanisms of action [3]. Briefly, it requires the presence of a photosensitizer able to absorb specific wavelengths and to react – upon illumination – with environmental oxygen molecules to produce singlet oxygen or other reactive oxygen species: these species are highly aggressive towards cellular components and can induce extensive photodamage, leading to cell death. PDT normally requires administration of the photosensitizer (or precursor) as a drug, but additional benefits arise when photoactive molecules are endogenously produced by the target pathogen, as in *Helicobacter pylori* (Hp). Hp can easily infect the gastric mucosa and can be responsible for several diseases in humans; notably, it spontaneously produces photoactive porphyrins [4] that can be exploited to induce photokilling by aPDT. Standard aPDT applied to Hp infections employs endoscopic fiber-optic devices – hardly tolerated by patients – for the intragastric delivery of light. In the context of the project CapsuLight,[2] which aims at the creation of an alternative intragastric device in the form of an ingestible LED-equipped pill, a customized illuminator (LED-BIP) was developed to perform *in vitro* irradiation tests on bacterial cultures [5]. In this paper we report about temperature measurements taken inside LED-BIP; the purpose of this work is to determine the best illumination parameters to irradiate bacteria while avoiding non-physiological heating of the samples, which would alter the response of the forthcoming survival tests on Hp.

Methods

Illuminators

A LED board is coupled to a programmable electronic board that controls up to 8 independent LEDs (Nichia Corporation Ltd, Tokushima, JP); two lithium batteries (CR2, 3 V) provide suitable power according to experimental needs, and the desired control current can be set *via* software before the experiment, as well as the number and operation time of each LED. Three LED boards were tested: violet (405 nm), green (500 nm) and red (630 nm). The assembled electronic elements are placed inside a photoabsorbent case (4 × 4 × 5.5 cm) and the sample dish is placed at a

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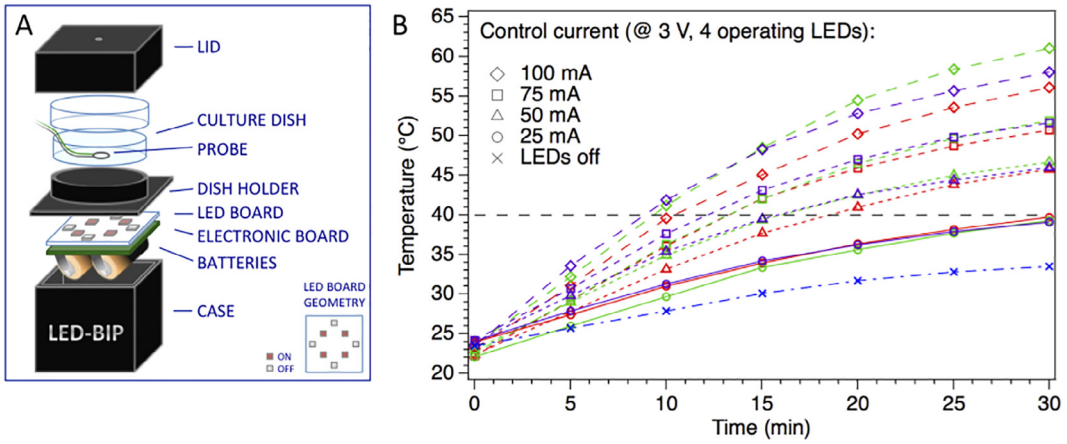

Fig. 1. A: Schematics of LED-BIP. B: Temperature *vs* time plots for the irradiation experiments with 4 LEDs. Colors of markers and curves correspond to colors of the LEDs (violet, 405 nm; green, 500 nm; red, 630 nm; blue, LEDs off). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Total irradiance of 4 simultaneously operating LEDs at different control currents and constant potential of 3 V and limit irradiation times (in brackets) with light doses obtainable from the different colors (final temperature of the liquid: max 40 °C).

Wavelength	Total irradiance [mW/m^2]				Maximum light dose [J/cm^2]			
	25 mA	50 mA	75 mA	100 mA	25 mA	50 mA	75 mA	100 mA
405	$8.64{\cdot}10^4$	$2.12{\cdot}10^5$	$3.10{\cdot}10^5$	$4.07{\cdot}10^5$	15.5 (30 min)	20.4 (16 min)	22.3 (12 min)	22.0 (9 min)
500	$9.67{\cdot}10^4$	$1.97{\cdot}10^5$	$2.72{\cdot}10^5$	$3.53{\cdot}10^5$	17.4 (30 min)	18.9 (16 min)	21.2 (13 min)	19.0 (9 min)
630	$7.69{\cdot}10^4$	$1.57{\cdot}10^5$	$2.37{\cdot}10^5$	$3.13{\cdot}10^5$	13.8 (30 min)	17.0 (18 min)	18.5 (13 min)	18.8 (10 min)

distance of 1 cm from the LED board thanks to a ring-shaped holder (Fig. 1A).

Irradiance measurements

LED irradiances were measured using a light spectroradiometer (SAMA Tools by S.A.M.A Italia Srl, Lucca, IT). Light sources were placed at a distance of 1 cm from the sensor.

Temperature tests

Conditions for the illumination tests were set to simulate the target experiment with Hp, which requires a microaerobic environment and incubation at 37 °C. A 35 mm culture dish (EuroClone SpA, Milan, IT) containing 2 ml of PBS buffer was then placed inside LED-BIP equipped with the desired LED board (violet, green or red); a thermocouple probe (Tastotherm MP-1300-D, IMPAC Electronic GmbH, Frankfurt, GE) was soaked in the buffer to monitor temperature changes. In order to change the light dose per time unit, illumination was performed applying different control currents to 4 simultaneously and continuously operating LEDs; this configuration ensures a homogeneous illumination of the dish. The illuminator was placed inside a sealed plastic bag mimicking microaerobic conditions and incubated at 37 °C.

Results and conclusions

Preliminary tests revealed that the LEDs keep stable during the experiment and that their irradiance output decreases only by 3% per hour of continuous operation. The maximum acceptable temperature for the buffer was set to 40 °C, since heating up to this limit would not alter the outcome of the photokilling effect [6], normally detectable for light doses between 5 and 40 J/cm^2 [7]. Results showed that the final temperature mainly depends on the control current and not on the LED color, as expected since LED specifications report a similar efficiency in terms of light/heat ratio for the three different wavelengths. Moreover, Fig. 1B shows that LEDs operated at a control current of 25 mA can be used for irradiation experiments lasting up to 30 min. Higher control currents require shorter irradiation times (Table 1), as extrapolated from the experimental data by polynomial fitting of the temperature values and from the irradiance values shown in Table 1, where the corresponding light doses are also reported. These results will drive the choice of the time/dose parameters to be applied in tests on living bacteria.

LED-BIP is an easy tool for the determination of the best irradiation conditions to be implemented in the design of the ingestible pill. Notably, it can also prove useful to perform future irradiation experiments on bacterial cultures and biofilms under controlled conditions to assess the efficacy of novel antimicrobial photodynamic therapies.

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