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Research Article

Light-Emitting Diode-Based Illumination System for *In Vitro* Photodynamic Therapy

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The aim of this study is to develop a light-emitting diode- (LED-) based illumination system that can be used as an alternative light source for *in vitro* photodynamic therapy (PDT). This illumination system includes a red LED array composed of 70 LEDs centered at 643 nm, an air-cooling unit, and a specific-designed case. The irradiance as a function of the irradiation distance between the LED array and the sample, the homogeneity and stability of irradiation, and the effect of long-time irradiation on culture medium temperature were characterized. Furthermore, the survival rate of the CNE1 cells that sensitized with 5-aminolevulinic acid after PDT treatment was evaluated to demonstrate the efficiency of the new LED-based illumination system. The obtained results show that the LED-based illumination system is a promising light source for *in vitro* PDT that performed in standard multiwell plate.

1. Introduction

Photodynamic therapy (PDT) is an emerging, minimally invasive therapeutic procedure that can selectively destroy the tumor tissue with photosensitizers activated by specific-wavelength light in the presence of oxygen [1, 2]. Upon absorption of the light, the photosensitizer initiates photochemical reactions that result in formation of reactive oxygen species (ROS), particularly singlet oxygen ($^1O_2$), which can cause significant cytotoxicity leading to cell death by apoptosis or necrosis within the target tissue [3, 4]. It is widely known that the irradiated light is one of the primary components of PDT, and thus the choice of light sources is crucial for PDT studies [5].

As for the PDT treatments, a stable, wavelength-specific, homogeneous, and large-area illumination is badly needed. Nowadays, a wide range of laser and nonlaser light sources have been used for PDT [5–9]. Laser light sources are not only very expensive, but also a specifically tailored optical system is required to expand the beam for the irradiation of large area. In particular, for the *in vitro* PDT measurement that performed in the standard multiwell plate, each well has to be irradiated one by one, which is a time-consuming experiment for PDT studies. The nonlaser light sources such as the conventional lamps (e.g., conventional tungsten filament and xenon arc lamps) can be used in conjunction with the optical filters to output specific wavelength for treatment of larger area [2, 6]. However, the irradiation devices based on the conventional lamps may lead to significant thermal effect, which should be avoided during PDT treatments [5, 6].

With the recent developments in high-power light-emitting diodes (LEDs), LEDs have been used as an alternative light source for PDT [5–11]. Compared to other available light sources for PDT, the advantages of LEDs include less expensive, less hazardous, thermally nondestructive, and readily available [11–13]. Moreover, LEDs can be arranged in arrays flexibly to irradiate large area according to the geometry of target area [2, 6, 12]. In this study, an LED based illumination system was developed, and the major properties of the illumination system, such as the irradiance as a function of the irradiation distance between the LED

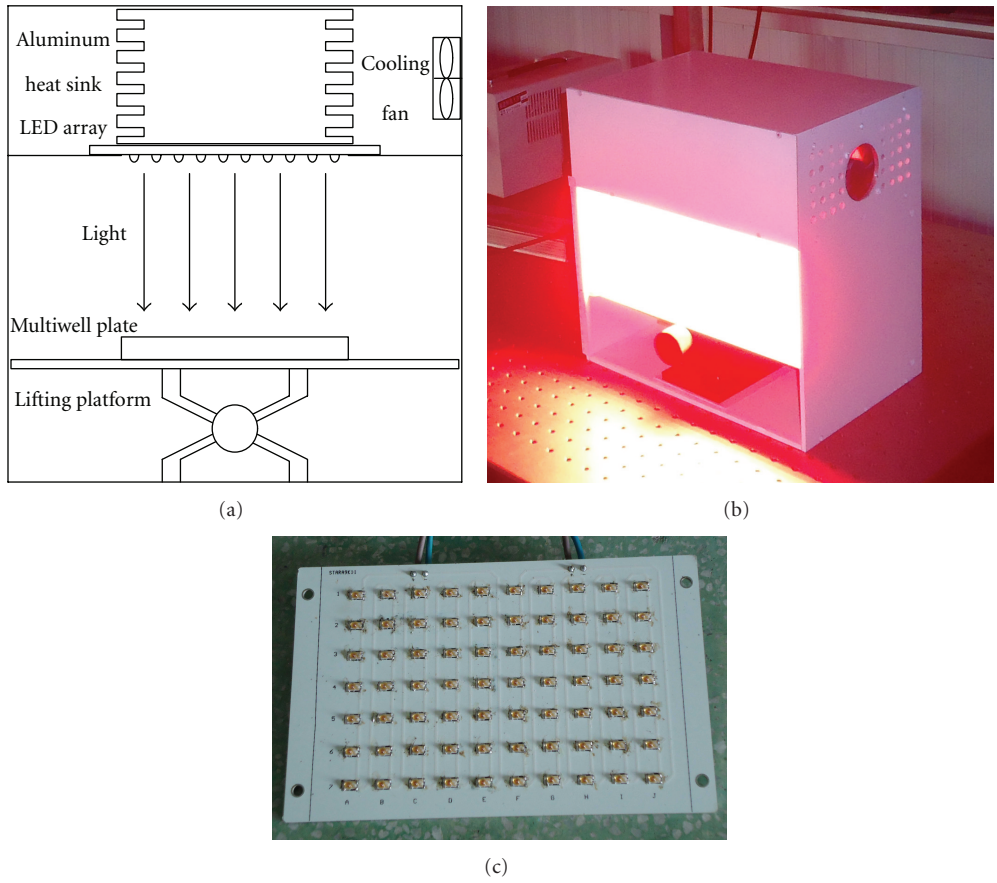

Figure 1: (a) Schematic diagram of the red LED based illumination system. (b) Photograph of the red LED based illumination system for PDT studies. (c) Prototype of the 10 × 7 LED array.

array and the sample, the homogeneity and stability of irradiation, and the effect of long-time irradiation on culture medium temperature, were characterized. In addition, in order to evaluate the efficiency of the illumination system, the survival rate of the CNE1 cells that sensitized with 5-aminolevulinic acid- (5-ALA-) mediated PDT was determined.

2. Experimental Section

2.1. Apparatus Design. A compact LED array-based illumination system with a homogeneous illumination area was proposed specifically for *in vitro* PDT, which usually perform for the cells grown in the standard multiwell plates (6-, 12-, 24-, 96-, or 384-well). The illumination system mainly includes a LED array and a specific case, and the LED array and the standard multiwell plate are both placed into the case. The arrangement of the LED array can be adjusted according to the distance between the LED array and the standard multiwell plate. In order to achieve the desired irradiance and homogeneity for *in vitro* PDT in standard multiwell plates, geometric optics simulation was conducted to optimize the design parameters (e.g., the arrangement of the LED array and the size of the case) for the proposed illumination system. The performances of the illumination system were characterized by evaluating the average irradiance produced on the area of the standard multiwell plate and the corresponding spatial non-homogeneity, which can be defined as the irradiance varied within the target area.

2.2. Construction of the Illumination System. As shown in Figures 1(a) and 1(b), the proposed illumination system includes a red LED array, an air-cooling unit and a specificde-signed case. The LED array composed of 70 LEDs (LXML-PD01-0040, LUXEON Rebel, Philips Lumileds lighting co., San Jose, CA, USA) in a 10 × 7 arrangement, which was illustrated in Figure 1(c), and the distance between the LEDs was 13 mm. Each single LED was soldered into a standard printed circuit board (size 16.3 × 10.3 cm, Fujian Xiangyun Photo-electric Technology Co., Ltd, Fuzhou, China) and connected in series. The LED array was mounted on the interlayer in the custom-designed case that fabricated in aluminum alloy (dimension 30.0 × 20.0 × 31.5 cm), and the LED array could be easily dismounted for exchange if needed. The air-cooling unit consisted of an aluminum heat sink and a cooling fan. In order to achieve the efficient heat transfer, the aluminum heat sink was attached tightly to the back surface of the printed circuit board, while the cooling fan was used to blow air for further dissipating heat. The standard multiwell plate containing samples was placed on a

lifting platform and can be irradiated directly under the LED array. The distance between the LED array and the lifting platform can be precisely adjusted in 1 mm steps vertically.

2.3. Measurement of the Spectrum and Irradiance of the Illumination System. The spectral emission for the red LED array was recorded with a spectrometer (USB4000, Ocean Optics inc., Dunedin, FL, USA) with a spectral resolution of approximately 0.2 nm in a range of 600–700 nm. Meanwhile, a laser power meter (FieldMaxII-Top, Coherent Inc., Santa Clara, CA, USA) with a 1.9 cm diameter circular effective sensing area was used to measure the irradiance.

2.4. Measurement of the Irradiance Homogeneity. The laser power meter was further used to measure the light distribution. The 1.9 cm diameter circular sensor defines the spatial resolution of each measurement. For determination of the homogeneity, the circular sensor was placed on a 1.9 $\times$ 1.9 cm grid, and the intensity was measured for each *x/y* position within the irradiated area.

2.5. Determination of Media Temperature during the Irradiation. 100 μL RPMI 1640 medium was put into each well of the 96-well plates to simulate the PDT *in vitro* experiment. The temperature of the culture medium was continually recorded by using a thermocouple data logger (TC-08, Pico technology Ltd., St Neots, Cambridgeshire, UK) over 20 min upon irradiation at room temperature.

2.6. Cell Lines and Culture Conditions. The CNE1 cell line was purchased from Guangzhou Taisheng Bio-Tech Co., Ltd. (Guangzhou, China). The cells were routinely cultured in RPMI 1640 medium supplemented with 10% new born calf serum (NBCS) and antibiotics (penicillin 200 U/mL and streptomycin 200 μg/mL), and the cells were maintained under the standard culture conditions at 37°C in a humidified 5% CO_2 incubator.

2.7. Cytotoxic Effect of In Vitro PDT. CNE1 cells were seeded into in a 96-well black wall/clear bottom costar plate (COSTAR 3603, Corning Inc., Corning, NY, USA) at a density of approximately 7500 cells per well and left to attach overnight. The medium was replaced with 100 μL serum-free culture medium containing 1 mM 5-ALA (Sigma-Aldrich, St. Louis, MO, USA) and incubated for 4 h. Then the cells were washed twice with PBS and re-fed with fresh culture medium. In order to obtain different light dosages for PDT evaluations, six optical attenuators (size 4 $\times$ 4 cm, Giai Photonics Co., Ltd, Shenzhen, China) with the transmissivity values of 0, 20%, 40%, 60%, 80%, and 100% were put onto the wells of the 96-well plate. Thereafter, the cells were illuminated for 20 min, while the control experiment representing the CNE1 cells incubated with 5-ALA but without light irradiation was performed in parallel. After the treatment, the cells were washed and were kept for 24 h under normal culture conditions for further evaluation of cell viability. Cell survival was determined by using standard MTT assay [14]. The optical density (OD) values were measured with the Mithras LB 940 plate reader (Berthold Technologies, Bad Wildbad, Germany) at 490 nm. Finally, the survival rate of the CNE1 cells under each attenuator was calculated by means of the following formula [15]:

$$\text{Survival rate (\%)} = \frac{\text{mean OD value of the irradiated cells}}{\text{mean OD value of the control cells}} \times 100. \quad (1)$$

2.8. Statistical Analysis. All the measured data were processed and analyzed by using OriginPro 8.0 software (OriginLab Corp., Northampton, MA, USA). Data were presented as means $\pm$ the standard deviation (SD) of three independent measurements.

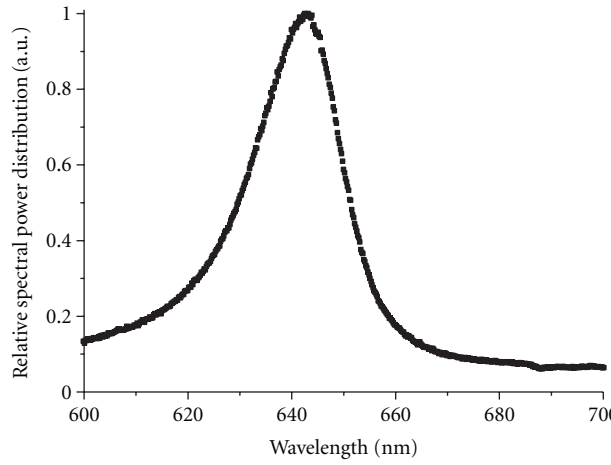

Figure 2: The normalized spectral emission of the LED array.

3. Results

3.1. The Spectrum and Irradiance of the LED-Based Illumination System. The normalized spectral emission for the LED array was shown in Figure 2. Peak power was found at the wavelength of 643 nm with a full width at half maximum (FWHM) of 21 nm. The irradiance emitted by the LED array is depending on the irradiation distance between the LED array and the irradiated sample. As shown in Figure 3, the irradiances were measured at five spots on the lifting platform for each irradiation distance by increasing the irradiation distance from 34 to 174 mm in 10 mm step. The irradiances decreased with the increasing of the irradiation distance and can be continuously adjusted in a range from 18 to 54 mW/cm^2. Moreover, the lower standard deviation of irradiances corresponding to better homogeneity can be found for the larger irradiation distance. Since the most commonly used irradiance is about 20 mW/cm^2 for *in vitro* PDT studies, the optimal irradiation distance can be determined to be about 164 mm for the present illumination system.

3.2. The Irradiance Homogeneity of the Treatment Area. When the irradiation distance was fixed at 164 mm, the irradiance distribution under the LED array was shown in

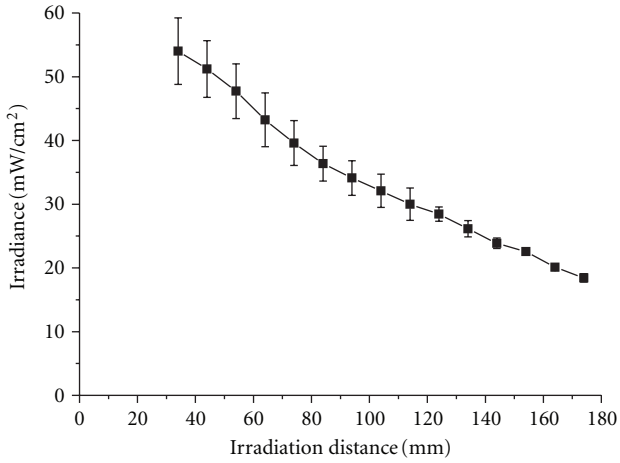

Figure 3: Irradiance as a function of the irradiation distance.

Figure 4(a). The irradiance was found to be good homogeneity within a field of the rectangular area (indicated by the solid line), which is approximately equal to the area of the standard multiwell plate. The average irradiance on the rectangular area was 18.6 mW/cm^2 with a standard deviation of 15%. In order to obtain different irradiances simultaneously, six optical attenuators with the transmissivity values of 0, 20%, 40%, 60%, 80%, and 100% were put onto the wells of the 96-well plate, and the irradiances of the six rectangular areas were shown in Figure 4(b). Different levels of irradiance can be simultaneously obtained with the optical attenuators, and the resulting average irradiances under the six attenuators were 0, 3.2, 7.0, 9.0, 12.7, and 20.3 mW/cm^2, respectively. Moreover, the irradiance almost remains constant during 20 min irradiation, while no significant warming of the LEDs can be detected (data not shown). The variance of irradiance under each attenuator was less than 10%, and there was no statistically significant difference for the cell viability between different wells under each attenuator, which can be found in the following PDT studies. As compared to the system that operates at a single fluence rate, this system would be very efficient for *in vitro* comparative PDT experiments. In particular, all the samples can be maintained in the same circumstance during the measurement.

3.3. The Media Temperature during the Irradiation. The temperature of the media increased when subjected to radiation, and the change in temperature of the culture medium used for *in vitro* PDT was monitored under the six different optical attenuators upon irradiation. In this study, the baseline temperature is about 37°C, which can be obtained directly from the measured prewarming media in the incubator prior to the light irradiation. Although the temperature of culture medium was increasing slowly upon the light irradiation, the increments were less than the maximum value of 1.5°C after 20 min, which caused no significant impact on PDT efficiency, as previously reported by Yang et al. [16].

3.4. CyTotoxic Effect of In Vitro PDT. The phototoxicity of 5-ALA-mediated PDT in CNE1 cells was assessed by MTT assay 24 h after PDT treatment. As shown in Figure 4(b), six different irradiances were simultaneously obtained by using the six optical attenuators. In this case, six different light doses (0, 3.8, 8.4, 10.8, 15.3, and 24.3 J/cm^2) were used for *in vitro* PDT treatment after 20 min of irradiation. The cell survival rates under the different light doses were shown in Figure 5. The survival rate of CNE1 cells was correlated well with the light dose, as expected. In the control groups, the cells incubated with 5-ALA for 4 h but without light irradiation showed no significant dark toxicity (data not shown).

4. Discussion

As for the *in vitro* PDT studies, a compact, low-cost, wavelength-specific, homogeneous, and large-area illumination system is widely desired. The traditional laser light sources can provide a monochromatic and very powerful illumination. Nevertheless, the main limitation of laser light sources is the limited irradiation area. Therefore, additional expanding optical systems are required to widen the beam, and each well in the standard multiwell plate has to be irradiated one by one, which is a relatively complicated and time-consuming experiment for PDT studies. Furthermore, the traditional laser light sources are relatively expensive, and careful maintenances are required. Compared to the laser light sources, the convenient lamps available for PDT have the advantage that they can be spectrally filtered to match the maximum absorption of any photosensitizers for treatment of large area [2, 6]. However, spectral filtering for lamps may lead to the dramatic fluence rates reduction. Additionally, in order to avoid significant thermal effects, the fluence rates have to be limited to the relatively low values [5].

In an effort to overcome the limitations of the current light sources applied in PDT treatments, we have successfully developed an LED array-based illumination system. The performance test suggests that the LED-based illumination system can provide a power-adjustable, wavelength-specific, homogeneous, and large-area illumination for *in vitro* PDT studies, and the survival rate of CNE1 cells was correlated well with the light fluence over a range of treatment conditions. Moreover, no additional optical system is required for achieving a homogeneous and large-area irradiation, which is convenient in operation. In addition, because of the availability of various wavelength LEDs, a LED-based illumination system with an appropriate wavelength can be readily developed as a light source to match the maximal absorption of the photosensitizers used in PDT studies.

5. Conclusions

A LED array based illumination system was successfully developed to provide low-cost, stable, power-adjustable, wavelength-specific, homogeneous, and large-area illumination specifically for *in vitro* PDT experiments. The irradiances can be continuously adjusted in a range from 18 to

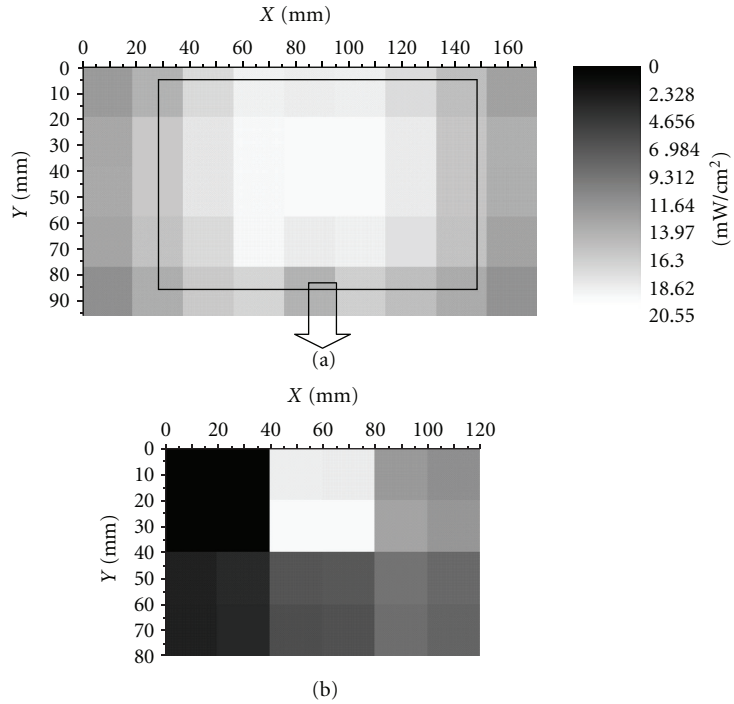

Figure 4: (a) Homogeneity of the irradiance for the irradiation distance of 164 mm between the LED array and treatment area, and the rectangular area is approximately equal to the area of the standard multiwell plate used for PDT studies. (b) Under the six different attenuators, the irradiances of the rectangular areas were 0, 3.2, 7.0, 9.0, 12.7, and 20.3 mW/cm^2, respectively.

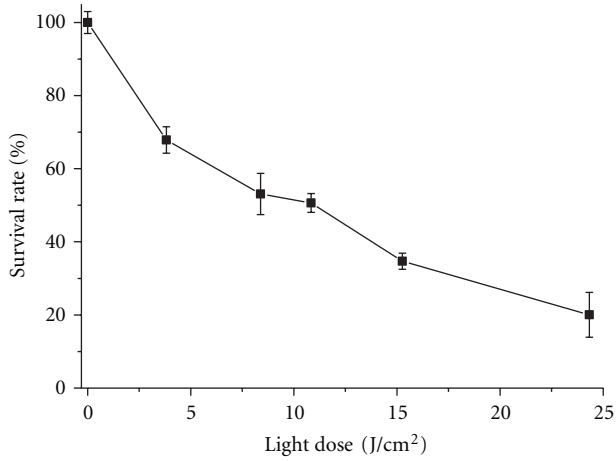

Figure 5: The cell survival rate of CNE1 cells after 5-ALA medicated PDT.

54 mW/cm^2 with an output wavelength centered at 643 nm. Furthermore, different irradiances can be simultaneously available for comparative PDT studies by using different optical attenuators. The efficiency of the illumination system was demonstrated by carrying out the 5-ALA-medicated PDT for CNE1 cells, and the obtained results suggest that the LED-based illumination system is a convenient and promising light source for PDT studies.

Acknowledgments

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