Investigation of Effective UVA1 Peak Wavelength Range to Application on Phototherapy

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Abstract

In present days, the phototherapy is a very effective method for treating most of the incurable skin diseases. A fluorescent bulb and xenon lamp are used as a conventional UV light source for this type of therapy. However, the normal part of skin is irradiated and the infrared radiation from the light source sometimes causes serious problems on the patient's health. For solving these problems a new UV light source is required. In previous study, we demonstrated in vivo experiment with mouse, that the group III nitride-based UVA1 light-emitting diodes (LEDs) were found very effective to treat only the part of skin diseases without infrared radiation. Recently, the wavelength range of phototherapy are using UVA1 (340-400 nm), however the effective wavelength range for treat skin diseases is remain unclear. In this study, to clarify the effective wavelength range, we developed an irradiation system which can emitting the peak wavelength of 365, 385 and 405 nm using UVA1-LED. Human T cell leukemia (Jurkat T cell) was irradiated with irradiation dose of 0, 10, 20 and 30 J/cm² to evaluate cellular apoptosis and necrosis induction in each peak wavelength. It was observed that the percentage of apoptosis increased as the irradiation dose increased in each peak wavelength. The most effective peak wavelength was 365 nm, which obtained 64, 84 and 98% of apoptosis induction on each irradiation dose. On the other hand, the percentage of necrosis induction was less than 1%. Effective peak wavelength on the UVA1 range to induce cellular apoptosis was examined by in vitro experiment. It was clearly that the peak wavelength 365nm have the potential to induce high apoptosis with very little necrosis. This study showed the possibility of UVA1-LED to application in the phototherapy.

Keywords: Light emitting diode; UVA1-LED; Phototherapy; Skin diseases; T cell; Apoptosis; Necrosis

Introduction

In present years, the ultraviolet irradiation for phototherapy is commonly used to treat refractory skin disease like an Atopic dermatitis, Psoriasis, Vitiligo and other else, when the internal and external medicine has no effect [1-3]. On the traditional phototherapy systems, xenon lamp, mercury lamp and fluorescent light bulb in combination with optical parts are using to obtain the adequate wavelength. Currently, there are two type of ultraviolet (UV) light use in phototherapy depending on the skin disease. The narrow-band UVB (wavelength: 311 ± 2 nm) is used to treat refractory skin diseases such as Psoriasis vulgaris, Vitiligo vulgaris, Prurigo nodularis and other else [4-6]. The UVA1 (wavelength: 340-400 nm) is used to treat Atopic dermatitis, Urticaria pigmentosa, cutaneous T-cell lymphoma and other diseases [7-9]. With the application of mercury lamp or fluorescent light bulb as the light source, large area and high irradiation intensity can be obtain, however there are some disadvantage as follows:

- Phototherapy systems using a mercury lamp or fluorescent light bulb require many square meters of floor space and consume approximately 3.5-5 KW of electricity.
- The light source has a relatively short lifetime.
- In case of mercury lamp the production and use will be prohibited after 2021 for contains toxic substance.
- Irradiation of healthy tissue is difficult to avoid when using a large-area irradiation device, leading to unwanted exposure.
- In the light source, some optical filter such as IR, blue and UV are used to obtain the UVB or UVA1 light; however the heat radiating from the light source is uncomfortable for the patients.
- Medical workers are also exposed to UV irradiation while operating the system.

UV1 therapies are used for the wavelength range of 340-400 nm; however it is not clarify the effective peak wavelength.

To solve these problems, the ultraviolet light emitting diode (UV-LED) based on an III-nitride semiconductor are considered adequate. The single chip of UV-LED is as small as 350 µm x 350 µm and can be operated using a dry battery [10]. The UV-LED has a narrow spectrum range and is rich in wavelength selectivity. Moreover, it is possible to irradiate only the disease part without heat radiation [11]. A visible LED has already been used to treat pimples and improve the skin quality [12,13]. However, the UV-LED has not been using until now in the phototherapy. To investigate the possibility of applying UV-LED in the phototherapy, in previous study we demonstrated that the UV-LED can irradiate only the disease part without heat radiation and other merit which is impossible done with UVA1-Lamp equipment [14]. Currently, in the UVA1 therapy uses broadband wavelength of 340-400 nm, however the effective peak wavelength is not clear yet. In this study, we would like to clarify the effective peak wavelength range of UVA1 (wavelength range: 340-400 nm) through evaluating the cellular apoptosis and necrosis induction by using UV-LED.

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Materials and Method

Development of UVA1-LED irradiation system

A UVA1 light irradiation system was developed with three different peak wavelength such as 365, 385 and 405 nm, with UVA1-LED. The light source in each wavelength, is composed for 10 pieces of high-power UVA1-LED (λ: 365 nm, FWHM: 12 nm; LZI-10UV00-0000, λ: 385 nm, FWHM: 13 nm; LZI-10UA00-U4, λ: 405 nm, FWHM: 17 nm; LZI-10UA00-U7 from LED Engin, Inc.) without optical lens and reflective plate. Figure 1 is showing the spectrum of each UVA1-LED wavelength. To obtain a high intensity of UVA1 light irradiation, the UVA1-LED was lined with the pitch of 1 cm. The light source is cooled by combination of heat sink and cooling fan. And the light source is controlled by a micro-computer which make it possible obtain CW or pulse output. The irradiation distance can be manually adjusted from 0 cm to 30 cm.

Evaluation of irradiation intensity and the irradiation uniformity

For evaluate the characteristic of the UVA1-LED irradiation system, the irradiation intensity and the uniformity of light source was measure. To evaluate the irradiation intensity, the light source was fixed above the center of a power-meter (PM100USB, S310C, Thorlabs, Inc.). The height of the light source was varied from 5 cm to 15 cm and the mean irradiation intensity was measured at 1 cm intervals. On the other hand, to evaluate the irradiation uniformity, the light source was fixed above the center of the power-meter, and the power-meter was moved at intervals of 1 cm while in the X axis direction and the Y axis direction from the center of the light source, and the irradiation intensity was measured in each place. The height of the light source was varied from 5 cm to 15 cm at 1 cm intervals. Figure 2a shows the result of the irradiation intensity. In the light source, optical lens or reflective plates are not used, for these reason the irradiation intensity decreasing when the irradiations distance increase. Figure 2b show the result of irradiation uniformity. The irradiation distance between 10 cm to 15 cm are shown similar uniformity on each wavelength. In this study, we apply the irradiation intensity from 10 cm to 12 cm depending the peak wavelength using on the biological experiment.

Evaluation of temperature characteristics of mediums

Two types of mediums: RPMI1640 (phenol red) Medium with L-glutamine and sodium bircarbonate (R8758, 599ML, SIGMA-ALDRICH Co.)/Fetal Bovine Serum (FBS) 10% (S1820-500, Biowest SAS) and Phosphate Buffered Saline (PBS) medium (Wako Pure Chemical Industries, Ltd.) were irradiate with the peak wavelength 365, 385 and 405 nm to analysis the temperature characteristics of the mediums. In the petri dish with diameters of 6 cm were injected 3 mL of each mediums and in the center of petri dish, a thermoelectric couple (TT-533, TANITA Corp.) was fixed. The light source was fixed above the center of the petri dish and the height was varied from 5 cm to 15 cm at 1 cm intervals. The temperature was measured on each height 10 minutes at 30 second interval. The experiment was done in ambient temperature average of 22.5deg C.

Figure 3 show the result of the temperature measurement. In comparison of their peak wavelength, 405 nm has obtained high temperature on the irradiation distance of 5 cm and 6 cm. That was observed on the RPMI1640 (phenol red)/FBS10% medium. On the other hand, in comparison of the both mediums, RPMI1640 (phenol red)/FBS10% medium was show approximately 0.5deg C higher in each condition than the PBS medium. It seems that the medium temperature increased for the light absorption occurred in phenol red. For this result, in the irradiation experiment to examine the effect of ultraviolet light in the leukemia cell, it was chooses to use PBS medium at the irradiation distance from 10 cm to 12 cm depending the peak wavelength.

Evaluation of cellular apoptosis and necrosis induction

To evaluate the effect of peak wavelength 365, 385 and 405 nm on the malignant cell, in this study Jurkat T cells (human T cell leukaemia, RCB 3052, RIKEN National Bio-Resource Center, Japan.) were applied. The Jurkat T cell it is leukemia cell which transfer from blood to the dermis and epidermis then cause cutaneous T-cell lymphoma [15]. Jurkat T cells were cultured with RPMI1640 (phenol red)/FBS 10% medium in 5% CO2 at 37deg C, 48 hours. In the irradiation experiment was used more than 85% live cells. Jurkat T cells were transferred to PBS medium and diluted to 0.5 × 10^6 cells/mL. 3 mL of Jurkat T cells was injected into the petri dish and irradiation dose of control, sham irradiation (Sham irradiation it is the test method for examine the effect of PBS medium to the cells. In this experiment, the cells soak in PBS medium was leave at room temperature the same irradiation time of 30 J/cm² without UVA1 light irradiation.), 10 J/cm², 20 J/cm², and 30 J/cm² UVA1 light irradiation were applied to three dishes. The UVA1-LED was fixed at a height were obtain 50 mW/cm² of irradiation intensity. Table 1 shows the experimental condition of each peak wavelength. After the irradiation, the cells was cultured for 24 hours in 5% CO2 at 37deg C, and then recovered the cells by trypsinization and centrifuged the cell pellet. The cells were stained with Cell-Max™ Annexin V-FITC and Live/Dead™ Green (Nippon Bussan, Co., Ltd.) and observed under fluorescence microscope (Olympus IX71, Olympus Co., Ltd.).
Figure 2b: Result of the irradiation uniformity measurement. The irradiation uniformity of light source was measured in X and Y axis direction from the centre of light source. The height of the light source was varied from 5 cm to 15 cm at 1 cm intervals. The irradiation distance between 10 cm to 15 cm are shown similar uniformity on each wavelength.

Figure 3: Evaluation of temperature characteristics mediums. Measurement of RPMI1640 (phenol red)/ FBS10% and PBS medium’s temperature at the irradiation distance from 5 cm to 15 cm. RPMI1640 (phenol red)/ FBS10% medium has shown in each peak wavelength, the light absorption which was arise from the phenol red containing medium. PBS medium shown low light absorption, and in the irradiation distance from 10 cm to 15 cm obtain near temperature of the ambient temperature (aver. 22.5deg C).

Results
Results of the cellular apoptosis and necrosis induction

Figures 4a and 4b shows the result of induction of cellular apoptosis and necrosis on Jurkat T cells (human T cell leukaemia) irradiated with peak wavelength 365, 385 and 405 nm. Figure 4a is the flow cytometry dot plots, which each dot means the condition of cell. The area Q1 defines necrosis, Q2 define apoptosis and Q3 define live cells. Figure 4b is the graph made with the values acquired by flow cytometry dot plots. In result, the average of live cells in control is 87.8% and in the

37deg C using RPMI1640 (phenol red)/ FBS 10% medium. Finally, the cellular apoptosis and necrosis was analyzed using a flow cytometric method. The cells were collected and centrifuged at 3,000 rpm for 2 min. at room temperature then 85 μL of binding buffer, 10 μL of annexin-V fluorescein isothiocyanate (FITC) and 5 μL of propidium iodide (PI) (Medical and Biological Laboratories Co. Ltd.) solution at 4deg C for 15 min. in the dark. Then, 400 μL of binding buffer was added to the cells and analyzed by flow cytometer equipment (BD FACS Aria II Cell Sorter, Becton Dickinson Co. Ltd.).
Test, n=3*P<0.01; **P<0.05). About the induction of cellular necrosis, there are not observed great differences on each peak wavelength and irradiation doses. In either case, necrosis induction was less than 1%. From those results define that the peak wavelength 365 nm is effective to induce specifically cellular apoptosis to human malignant T cells on the UV A1 light range. The induction of T cell apoptosis is mediated through activation of the FAS/FAS-ligand system in irradiated cells as a consequence of singlet oxygen generation. The generation of singlet oxygen is considered to have a central role in inducing apoptosis and is
an underlying mechanism of UV A1 phototherapy [16].

Discussion

For the treatment of refractory skin disease it is apply the UV A1 phototherapy method in present days. It is recognized safety in the UV A1 phototherapy, the wavelength range from 340 nm to 400 nm. However, the effective peak wavelength which can induce cellular apoptosis for acquire skin cure is not clear yet. In this study, we investigated the detail of the effective peak wavelength in UV A1 range using three peak wavelengths 365, 385 and 405 nm (UV A1-LED). Jurkat T cells used in dermatological research were used to investigate the effect only each peak wavelength. The irradiation experiments were conducted in a state of constant room temperature without radiation heat from the light source. The Jurkat T cells was irradiate soak in the PBS medium, which is transparent and prevent the light absorption. With each wavelength was irradiated with dose of 10, 20 and 30 J/cm² in the Jurkat T cells to analysis the induction of cellular apoptosis and necrosis. The apoptosis induction was increased significantly when the irradiation dose increased. The peak wavelength 365 nm was obtained high cellular apoptosis induction. In comparison with cell control which is not UV A1 light irradiate, obtained 64% of apoptosis induction on the irradiation dose 10 J/cm², 84% of apoptosis induction on the irradiation dose 20 J/cm² and 98% of apoptosis induction on the irradiation dose of 30 J/cm². On the other hand, necrosis induction was less than 1%. These results shows possible induction of apoptosis, which is necessary in the treatment and it, clarified the possibility of UV A1-LED to apply in the dermatologic therapy.

Conclusion

In this study, we developed a simple irradiation system which can emit three type of peak wavelength 365, 385 and 405 nm using UV A1-LED, to investigate the most effective wavelength range on the UV A1 range for induction cellular apoptosis. The Jurkat T cell was irradiated with irradiation dose 10, 20 and 30 J/cm² by using each wavelength mention above. At the results, the peak wavelength 365 nm was acquire high cellular apoptosis than the peak wavelength 385 nm and 405 nm, which achieve 98% of cellular apoptosis with 30 J/cm². At the comparison, in some irradiation dose 30 J/cm² the peak wavelength 385 nm was 76% and in the peak wavelength 405 nm was 52%. This is obvious that the effective peak wavelength on UV A1 range, and we found that the range 390 nm to 410 nm it is not very effective. For decades without the benefit of effective cure wavelength range has been used. There is no study before about this investigation in analysis of the detail effect of peak wavelength. In this study, we examine detail the effective wavelength range using the commercial UV A1-LED which is difficult to done with the conventional phototherapy system using lamp or fluorescent bulb. Through this investigation, we clarify that the wavelength range 360 nm to 390 nm is effective to induced cellular apoptosis with low cellular necrosis which produces adverse effect. This result shows basic develop of a safety phototherapy system for the next generation.

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References