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SHORT COMMUNTICATION

Development of a new water sterilization device with a 365 nm UV-LED

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Abstract Ultraviolet (UV) irradiation is an effective disinfection method. In sterilization equipment, a low-pressure mercury lamp emitting an effective germicidal UVC (254 nm) is used as the light source. However, the lamp, which contains mercury, must be disposed of at the end of its lifetime or following damage due to physical shock or vibration. We investigated the suitability of an ultraviolet light-emitting diode at an output wavelength of 365 nm (UVA-LED) as a sterilization device, comparing with the other wavelength irradiation such as 254 nm (a low-pressure mercury lam) and 405 nm (LED). We used a commercially available UVA-LED that emitted light at the shortest wavelength and at the highest output energy. The new sterilization system using the UVA-LED was able to inactivate bacteria, such as *Escherichia coli* DH5α, Enteropathogenic *E. coli*, *Vibrio parahaemolyticus*, *Staphylococcus aureus*, and *Salmonella enterica* serovar Enteritidis. The inactivations of the bacteria were dependent on the accumulation of UVA irradiation. Taking advantage of the safety and compact size of LED devices, we expect that the UVA-LED sterilization device can be developed as a new type of water sterilization device

Keywords UVA-LED · Disinfection · Sterilization · Bacteria

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1 Introduction

Sterilization technology is useful in various ways for our daily life. For example, it is used in water and sewerage systems and for foods and medicine. Methods widely used for sterilization include chemicals, heat, ultraviolet (UV) radiation, and ozone [5]. Chemicals (chlorine, peroxide, etc.) are used extensively for sterilization because of their simplicity; however, they can have some adverse effects, such as modifying the quality of the target. In addition, sterilization by chlorine has the drawback of generating odorous substances and biohazardous materials [1, 7].

Ultraviolet does not remain in water and has little influence on the environment. In addition, it does not produce drug-resistance to bacteria [10]. Thus UV sterilization performs effective disinfection without the addition of chemical substances and has recently been in the spotlight as a substitute for chlorination. Conventional methods for UV sterilization use UV lamps, such as low- and medium-pressure mercury-vapor lamps [7, 8]. These are used to sterilize workspaces and tools used in biological laboratories and medical facilities. UV lamps emit UV at a wavelength of 254 nm, which coincides very well with the peaks of the germicidal effectiveness curve (i.e. the effectiveness for UV absorption by DNA) [11]. UV at a wavelength of 365 nm is classified as UV-A (320–400 nm),

and the damaging potential of UV-A is lower than that of UV-C (100–280 nm) [2, 13]. An ultraviolet-light emitting diode (UV-LED) emits light at a wavelength of 365 nm, which is not as hazardous for human eyes and skin as the 254 nm wavelength lamp. Furthermore, the UV-LED does not contain mercury, so it does not have harmful effects on either the human body or the environment. A UV-LED is an environment-conscious sterilizer.

The mercury-vapor lamps contain mercury, which is toxic to the environment as well as to the human body [11]. In addition, sterilizers using UV lamps must be designed to suit the shape of the lamps, which are large in most cases and take up a lot of space. Therefore, new sterilization equipment of low energy consumption can be designed in various shapes and sizes without using harmful substances.

In this paper, we studied the possibility of using UV-LED for water sterilization. At present, the commercially available UV-LED model with the shortest wavelength is the UVA-LED, which emits light at a wavelength of 365 nm. We used the UVA-LED model that had the highest output power of those currently being developed. Currently, UV-LEDs that emit between 365 to 500 nm are mainly used for curing UV curable resins; therefore, to use an LED for sterilization is a novel approach. Using a UV-LED will not only provide an alternative to low-pressure mercury lamps, but will also provide the opportunity to develop small, space-saving sterilization equipment. Because of its compact size, UV-LED can sterilize small or narrow spaces, and it will provide opportunities to design many types of sterilizer for different purposes.

2 Method

2.1 Experimental device

We used a high-power UVA-LED (NCCU033(T); Nichia Corporation, Japan, wavelength 365 nm) to make the sterilization device. We connected eight UVA-LEDs in series, and applied a direct-current (DC) power supply (PAS40-9, Kikusui Electronics Corporation, Japan). The current was set at a constant current of 500 mA. The irradiation distance from the UV-LED to the bacteria was set at 20 mm. The top view of the experimental device is shown in Fig. 1a, the dimensions of the UVA-LED used for the experimental device are shown in Fig. 1b, the wavelength spectrum characteristics are shown in Fig. 1c, the radiation characteristics are shown in Fig. 1d, and light fluences versus time in Fig. 1e [6]. A total of 150 μl of the bacterial suspension was placed into each well of a sterilized 96 well plate (Falcon, Franklin Lakes, NJ, USA) and exposed to UV light.

An LED that emits light at a wavelength of 405 nm (prototype; Nichia Corporation, Japan,) and a low-pressure UV lamp that emits light at a wavelength of 254 nm (3UV Multi-Wavelength Lamp, 3UV-38; UVP, Inc. CA, USA) were used to compare the sterilization ability at different wavelengths.

2.2 Bacterial strains and growth conditions

Escherichia coli DH5α was purchased from Takara Bio Incorporated (Otsu, Japan). Enteropathogenic *E. coli* (EPEC) strain RIMD0509829, *Vibrio parahaemolyticus* strain RIMD2210633, and *Staphylococcus aureus* strain RIMD3112001, were obtained from Research Institute for Microbial Diseases, Osaka University, Japan. *Salmonella enterica* serovar Enteritidis was isolated from a patient with salmonellosis. Bacteria were cultured in Luria-Bertani (LB) broth (1 % tryptone, 1% NaCl, 0.5% yeast extract) at 37°C for 18 h. Cells were centrifuged (5,000 × *g*, 10 min, 4°C), washed three times with sterilized phosphate-buffered saline (PBS, pH 7.4), and suspended in PBS at an initial concentration of 5–7 × 10^4 CFU ml^{-1} or 5–7 × 10^6 CFU ml^{-1}. A total of 150 μl of the bacterial suspension was placed into each well of a sterilized 96-well plate (Falcon, Franklin Lakes, NJ, USA) and exposed to UV light. When estimating the effects of pH on the sterilization ability, the pH of PBS for making the bacterial suspension was adjusted to each of the pH by the NaCl or HCl.

2.3 UVA-LED irradiation

The distance between the UVA-LED and the surface of the bacterial solution was 20 mm. UVA-LED irradiation was performed in a dark room at 25°C for various time periods, and control samples were kept in a completely dark environment in the same room for the same period of time. When estimating the effects of the temperature on the sterilization ability, the equipments and the bacterial solution had been kept in each of the temperature from 1 h prior the experiments until finishing the UVA irradiation.

2.4 Determination of the inactivation level

The inactivation level was determined by a colony-forming assay. After UV irradiation, bacterial suspensions were diluted appropriately, plated on LB agar plates, and incubated at 37°C for 18 h. After incubation, the number of colonies was counted, and a log survival ratio or an

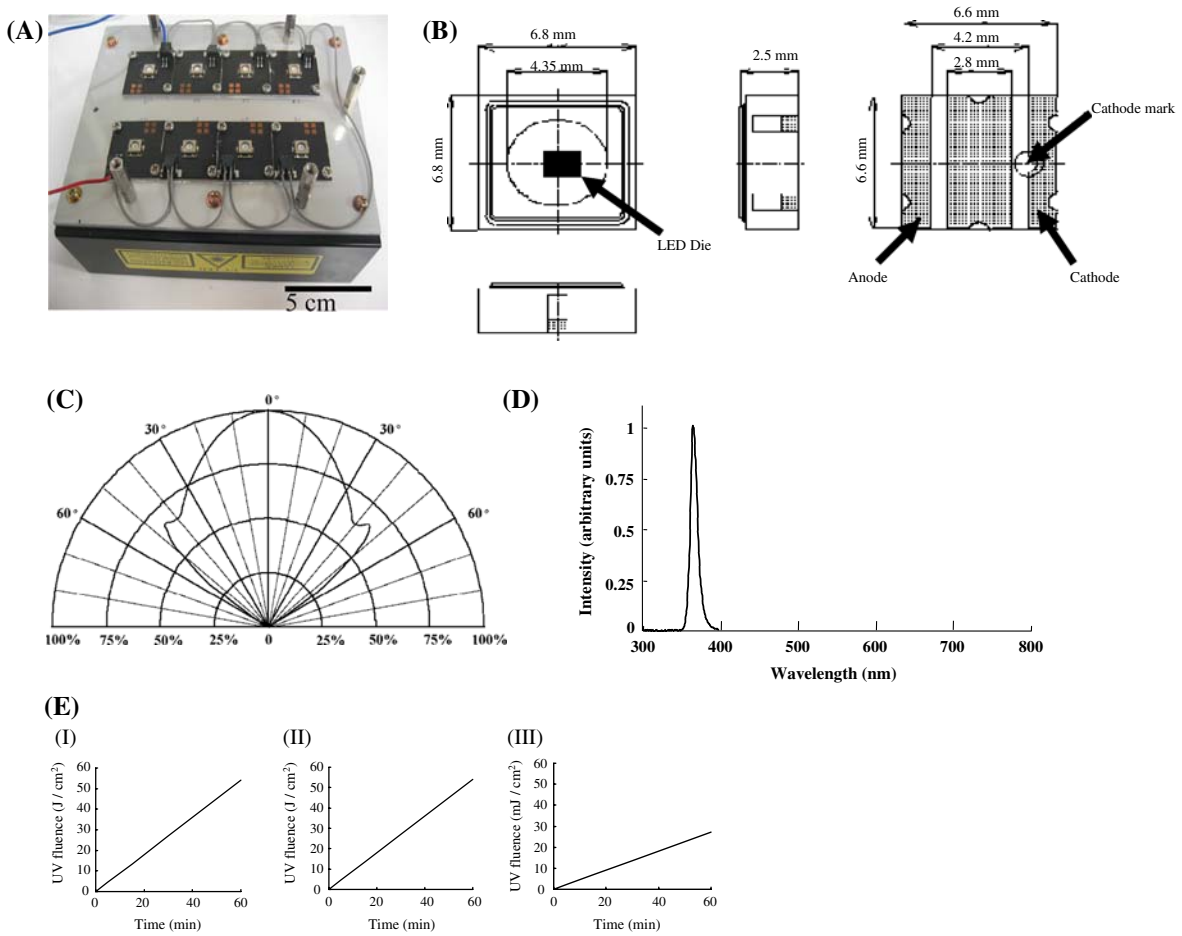

Fig. 1 Sterilization device. **a** *Top view* of the experimental device. **b** Dimensions of the UV-LED. **c** The wavelength spectrum of the UVA-LED. **d** Radiation characteristics of the UVA-LED. **e** Light fluences versus time. (*I*) 360 nm-LED fluences, (*II*) 405 nm-LED fluence, (*III*) 254 nm- low-pressure UV lamp fluence

inactivation percentage was calculated using the following equation:

$$\log \text{ survival ratio } = \log (\text{Nt}/\text{N0})$$

where Nt is the colony count of the UV irradiated sample, and N0 is the colony count of the sample before UV irradiation.

3 Results and discussion

3.1 The relation between the UV irradiation time and the inactivation rate

We estimated the ability of UVA-LED light to inactivate *E. coli* DH5α (Fig. 2). At 54 J/cm^2 of UVA irradiation, the inactivation efficiency reached a maximum $\log_{10}$ reduction of 3.9. These data indicate that UVA-LED can inactivate bacteria in water.

3.2 Pulse irradiation and continuous irradiation

The sterilization rate using pulse irradiation and continuous irradiation was compared used by *E. coli* DH5α (Fig. 2). We irradiated using a 1 A current pulse: 10 ms ON and 100 ms OFF (duty ratio 1/10). Under continuous irradiation, the current was set to 500 mA as described in the experiment above. Applying such high currents to UV-LEDs can generate high amounts of heat, and can cause problems with the experimental device; to prevent this, pulse irradiation must be performed, particularly when using 1A currents .

The inactivation rate was 100% for an irradiation dose of 27 J/cm^2 at both 500 mA continuous irradiation and 1 A pulse irradiation. At low irradiation doses, a continuous irradiation of 500 mA was sufficient to inactivate the bacteria, whereas pulse irradiation was not. However, the advantages of using pulse irradiation include the prevention of heat generation and the capability to use higher outputs of irradiation, which penetrate deeper into the

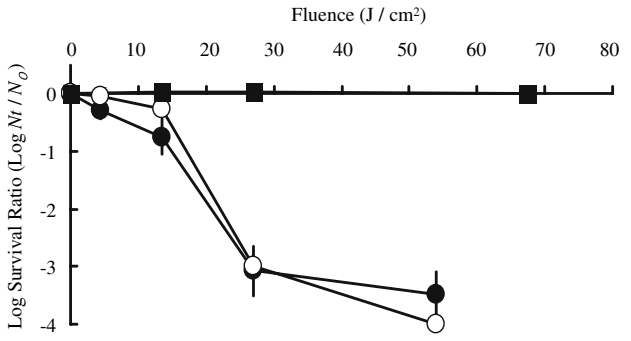

Fig. 2 UVA-LED irradiation inactivates *E. coli* DH5α in a UVA dose-dependent manner. The initial number of cells was 10^4 CFU ml^{-1}. The log survival ratio is described in the Materials and methods. (*filled circle*); Consecutive irradiation of *E. coli* DH5α by UVA-LED. The current was set at 500 mA as described in the text. (*open circle*); intermittent irradiation of *E. coli* DH5α by UVA-LED. The bacteria were irradiated with 10 ms 1A pulses with 100 ms between each pulse (duty ratio 1/10). (*filled square*); Non-irradiated control samples (in the dark at 25°C). The data represent means ± SD (n = 5)

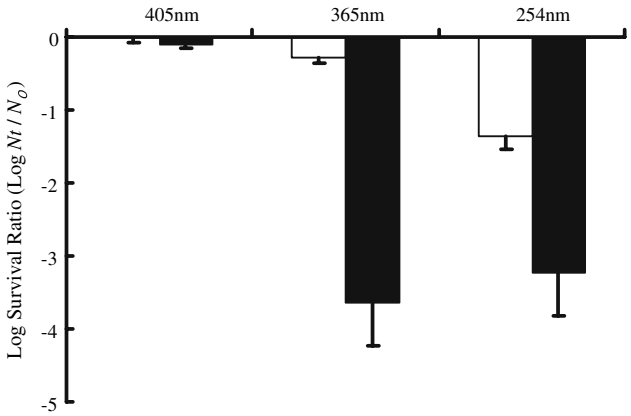

Fig. 3 The log survival ratio depends on different wavelengths of irradiation. The initial number of *E. coli* DH5α was 10^4 CFU ml^{-1}. Log Survival Ratio is described in the Materials and methods. A total of 405 nm wavelength of light emitted by an LED (prototype; Nichia Corporation, Japan,) and 254 nm wavelength of light emitted by a low-pressure UV lamp (3UV Multi-Wavelength Lamp, 3UV-38; UVP, Inc. CA, USA) were used to compare the sterilization ability of different wavelengths of light. White colum; 15 min exposured of each of light. Black column; 30 min exposure of each of light. The data represent means ± SD (n = 5)

sample as compared to lower outputs. Therefore, it is beneficial to use pulse irradiation for sterilization. For the remaining experiments, we performed sterilization using pulse irradiation.

3.3 Comparison of the sterilization ability by wavelength

In order to evaluate the sterilization ability of 365 nm UVA-LED, we conducted an experiment using an LED that emits light at 405 nm and a low-pressure UV lamp that emits light at 254 nm used by *E. coli* DH5α (Fig. 3). No sterilization effect was observed by irradiating with light at 405 nm. This suggests that the sterilization effect of the UVA-LED is due to light emitted at a wavelength of 365 nm. Indeed, similar sterilization abilities were observed with the 364 nm wavelength UVA-LED and the 254 nm wavelength UV lamp which sterilized at a significantly low energy (J/cm^2). Although a UVA-LED can sterilize at an irradiation dose of 27 J/cm^2, it can not sterilize at irradiation doses as small as those used by low-pressure UV lamps.

3.4 Effects of temperature and pH of the water on inactivation percentage

Next, we estimated the effect on the environmental condition, such as temperature (Fig. 4a) and pH (Fig. 4b) of the bacterial suspension, because there were reported that temperature and pH effect on sterilization [3, 4, 9]. Sterilization abilities were indicated higher at 20°C and the pH 8. Moreover, different bacteria, such as *E. coli* DH5α, Enteropathogenic *E. coli*, *V. parahaemolyticus*, *S. aureus*, and *S. enterica* serovar Enteritidis had each of different sensitivity for temperature and pH (data not shown), it will be necessary to consider sensitivity for each of bacteria.

The purpose of this research was to investigate the possibility of sterilizing by using a UVA-LED and to determine the possibility of applying UVA-LED to a sterilization device. From our results, we show that irradiating with a UVA-LED for about 30 minutes can almost completely sterilize nonpathogenic and pathogenic bacteria. It is difficult to instantly sterilize as with UV lamps, however, the bacteria were fully sterilized by a UVA-LED sterilizer after a certain time. In a water drinking guideline by World Health Organization, *E. coli* or thermotolerant coliform bacteria must not be detectable in any 100 ml sample in the bacteriological quality of drinking water [13]. Thus, it will be possible to use this system for drinking water sterility. It is hard to estimate "suitability" for animal or human consumption or surgery, because there are a lot of factors included for estimating the suitability, such as taste, a smell, an appearance, not only bacterial existence. Thus, we need future experiments about estimating suitability of this irradiated water for animal or human consumption or surgery.

Compared to the low-pressure UV lamp, a UVA-LED is considerably smaller and operates at a higher intensity, which would make it more useful over a broader range of applications. In addition, 365 nm UV can penetrate further than 254 nm UV. Therefore, it is conceivable that 365 nm

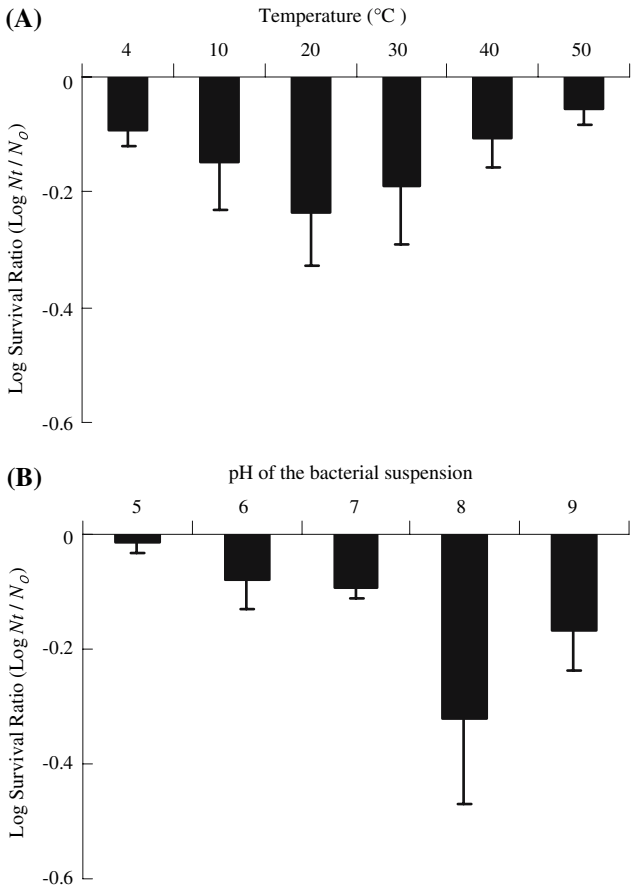

Fig. 4 Effects of the environmental condition on sterilization ability. The initial number of *E. coli* DH5α was 10^4 CFU ml^{-1}. Log survival ratio is described in the Materials and methods. Consecutive irradiation of *E. coli* DH5α by UVA-LED for 15 min. The current was set at 500 mA. **a** Temperture dependency of sterilization ability. The equipment and the bacterial solution had been kept for 1 h prior to the experiment. The data represent means ± SD (n = 5). **b** pH dependency sterilization ability. The pH of PBS for making the bacterial suspension was adjusted to each of indicated pH by the NaCl or HCl. The data represent means ± SD (n = 5)

UV is more effective than 254 nm UV for sterilizing cloudy or colored water.

Because the UVA-LED disinfection system studied here was a small, ELISA plate system, challenges will be encountered when trying to apply this system to a large volume of flowing water. Because much of the LED light leaked out of the ELISA device used this study, most of the light from the LED was not used for inactivating the bacteria. Clearly, the geometry of the light source will need to be adapted to develop an effective device for a larger-scale UVA-LED water disinfection system.

In the future, we will conduct experiments using larger volumes of water to develop UVA-LED into practical use in a circulating water system to take advantage of the safety and compact size of UVA-LED sterilization devices.

4 Conclusions

We studied the possibility of using commercially available UVA-LED model for water sterilization. UVA-LED is capable of sterilization and should be available for use in sterilization equipment.

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