UVA-LED Air Disinfection

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We developed a UVA-LED device capable of emitting germicidal radiation at 365 nm wavelength. Its germicidal activity was previously tested over a wide range of microorganisms in suspensions and on surfaces. In this study, we examined UVA-LED bactericidal activity in air, first using the model organism *Escherichia coli* but later extending our observations to ask if the technology provided germicidal effects against the real airborne pathogens *Serratia marcescens* and *Micrococcus luteus.* In a preliminary study, we observed 99.9% reduction of *E. coli* populations after 75 min of exposure to high power UVA-LED under constant current (15.3 W/cm^2) or pulsed current (1.3W/cm^2). In an extended study, UVA-LED reduced cell viability in aerosols of *S. marcescens* and *M. luteus* by more than 90% after 10 min of constant exposure (37.2 W/cm^2) or a pulsed protocol (7.4 W/cm^2). Further studies are in progress to examine the UVA-LED germicidal effect on viruses and airborne allergens. We suggest that UVA-LED irradiation will have advantages for air disinfection in real-world situations.

Keywords: Ultraviolet-A light emitting diodes (UVA-LED); airborne bacteria; Ultraviolet germicidal irradiation (UVGI); Bacterial bioaerosol

1. Introduction

Since the industrial revolution there has been a need for efficient air disinfection and sterilization technologies, due to increasingly high levels of air pollution and the stresses and diseases caused by them. Several pollutants from indoor sources affect human health [1]. The main technologies used to clean indoor air are ventilation, filtration, ultraviolet germicidal irradiation (UVGI) and carbon adsorption. Other technologies still in development include photo catalytic oxidation (PCO), ionization, pulsed UV light, pulsed electric fields, ozone generators, microwave radiation, plasma fields, gamma irradiation and impregnated filters [2). UVGI has proven to be the most effective against indoor bio-pollutants [3], [4], [5], and [6]. In this study we aimed to combine in one device the effectiveness of UVGI against indoor air bio-pollutants with the advantages of LED technology; the UVA-LED device is capable of emitting a UVGI of the wavelength 365 nm (*i.e.,* the UVA band). The effectiveness of UVA-LED in disinfecting suspensions and surfaces has been proven in prior work by our research group [7], [8], [9], and [10].

2. Experimental section

To evaluate UVA-LED bactericidal activity in air, two experiments were successfully conducted: the first with the model organism *Escherichia coli*, the second with the known airborne contaminants *Serratia marcescens* and *Micrococcus luteus*.

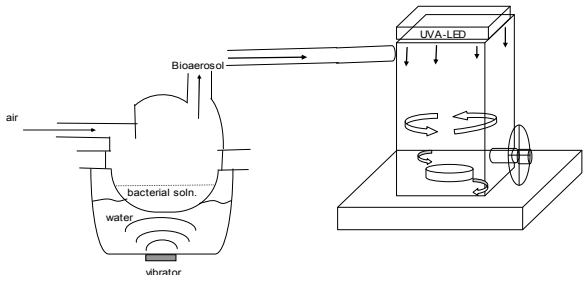

Fig.1 Generation of a bioaerosol, mixing it with air, and exposing it to UVA-LED in the aerosol chamber**.** Arrows in the box on the right represent maintenance of the atmosphere by two fans at the bottom of the box.

2.1 Estimation of the bactericidal effect of UVA-LED in air

2.1.1 Bacterial sample preparation

E. coli strain DH5α was purchased from Takara Bio Inc. (Otsu, Japan). Samples were prepared as previously described [11]. Briefly, stationary phase *E. coli* cells grown on a Luria-Bertani agar plate (LB plate) were used to resist the effect of the ultrasonic wave during aerosol generation using the ultrasonic Nebulizer [1], [20]. *E. coli* cells cultured overnight in LB broth were collected by centrifugation for 3 min at 10,000 rpm and 4° C, washed twice with phosphate buffered saline (PBS, pH 7.4), and adjusted to an optical density of 1.0 at 600 nm for a bacterial concentration of 2×10^8 colony forming units (CFUs) / ml.

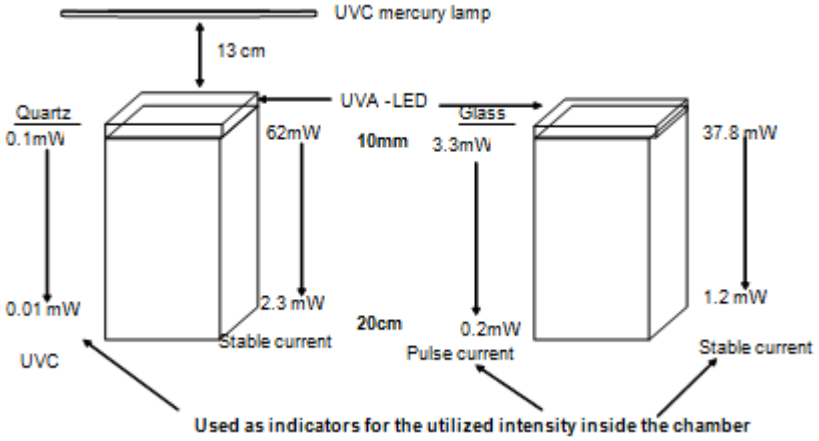

Fig.2 Components of the irradiation apparatus and measured radiation intensities using UVA-LED (either stable or pulsed current) or UVC mercury lamp with quartz or glass covers.

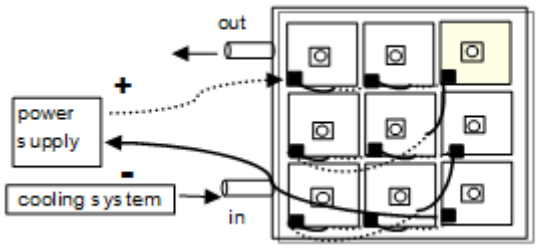

Fig.3 Diagram of the UVA-LED showing the [Editors Note: dimensions in terms of lengths are not shown] of each LED, the electrical circuit and the cooling system. Each of the nine small boxes represents one LED.

2.1.2 UVA-LED or UVC exposure and bioaerosol sampling

Aerosols containing *E. coli* cells, produced by an ultrasonic Nebulizer (NE-U17, Omron Co. Ltd., Kyoto, Japan), were exposed to UVA irradiation emitted from a 9-LED device (NCSU033E, Nichia Corp., Tokushima, Japan) positioned in an acrylic test chamber (2 L volume); the bioaerosol inside the chamber was maintained by two fans (powered by 1.5 V batteries) placed at the bottom of the chamber (Fig. 1). LB plates (5 cm dia.) at the bottom of the chamber remained covered by their lids during UV exposure time. Lids were removed for two min for bioaerosol sampling after UVA-LED exposure ended. Fig. 2 shows the dimensions of the test chamber and the measured UVA intensities inside, comparing exposure using a glass (UVA-LED) with that of a quartz cover for UVC low mercury lamp (UVP, Upland, CA).

2.2 Bactericidal effect of UVA-LED on airborne bacteria in an actual air stream disinfection system

Based on experimental designs described previously [12], [13], we built a continuous flow airstream disinfection system with accurate bioaerosol sampling and capable of measuring bactericidal activity of UVA-LED on airborne microorganisms; the apparatus also controlled air-flow, RH, and temperature. The system comprised four units, pure air

supply unit, Bioaerosol generation and RH regulation unit, UVA-LED exposure unit, and Bioaerosol sampling unit. Tow strains of airborne bacteria used in the current study, *Serratia marcescens* JCM 1239 and *Micrococcus luteus* (IFO3333). Procedures were done as reported previously [14] (data not shown). After UVA-LED exposure, the surviving fraction of the bioaerosol was determined by two stage bioaerosol impactor, flowing at a velocity of 10 LPM.

The following equations were used to address and analyze the results;

Log survival ratio = Log N_1/N_0 (1)

Where N_1 represents the number of CFU at a certain time and N_0 represents the number of CFU at time 0.

Total output energy $E_{out} = \eta I_0 T$ (2)

Where η represents the number of LEDs used in the experiment and I_0 is the output energy of the single UVA-LED in mW and T is the time of UVA-LED exposure in seconds.

The total utilized energy $E_{ut} = I_1 T \times A$ (3)

Where I_1 represents the measured average UVA intensity (mW/ cm^2) inside the stainless steel test chamber at 0.5 cm from the glass cover; T represents the total exposure time in seconds and A is the total exposed area of the glass cover to the UV irradiation in cm^2.

Percentage of the utilized energy to the output energy was calculated from the following equation;

$E_{ut} / E_{out} \times 100$ (4)

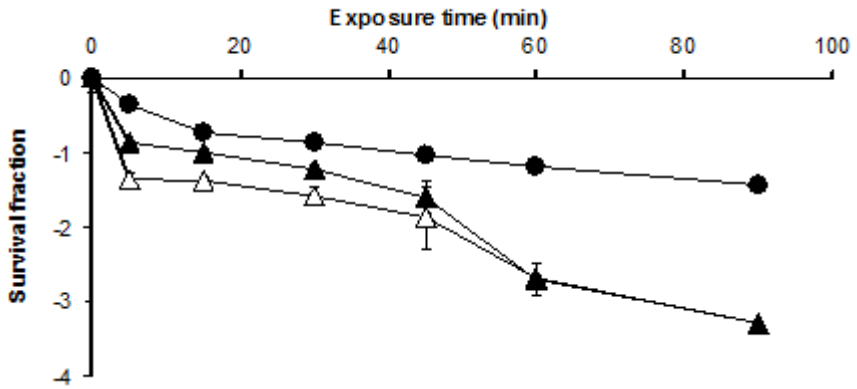

Fig.4 Survival curves of *E. coli* DH5α after exposure to stable or pulsed UVA-LED. Closed circles: control; closed triangles: pulsed UVA exposure; open triangles: stable UVA exposure. Data are expressed as means ± SD of three independent experiments**.**

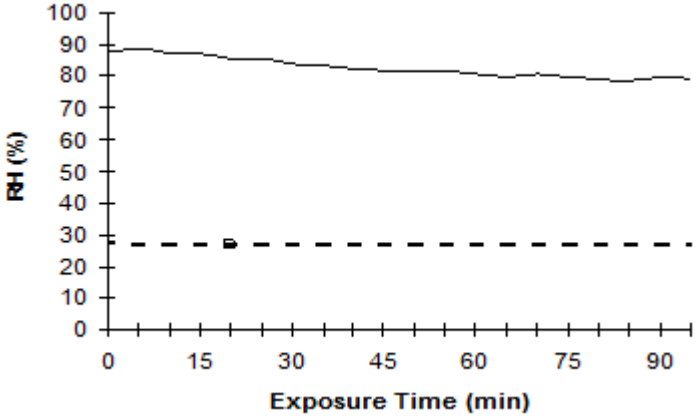

Fig.5 Temperature (dashed line) and relative humidity (solid line) measured concurrently during exposure of *E. coli* to UVA-LED.

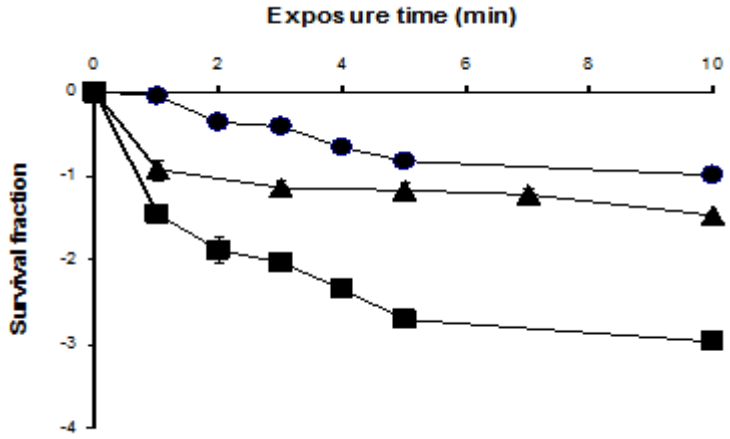

Fig.6 Survival curves of *E. coli* DH5α after exposure to UVC (0.01 mW/cm^2). Circles: control: triangles: stable UVA exposure; squares: UVC exposure. Data are expressed as means ± SD of three independent experiments.

Table.1, summary of the utilized and wasted energy during UVA-LED or UVC exposures in the *E. coli* experiment

	Time consumed to perform -3 Log reductions(min)	Total utilized energy (W/cm^2)	% of the utilized to the output energy
UVA-LED Stable current (37.8mW/cm^2)	75	15.3	25%
UVA-LED Pulse current (3.3mW/cm^2)	75	1.3	11%
UVC (0.1mW/cm^2)	10	0.087	5%

3. Results and Discussion

In the first experiment (Fig.4 and Table 1), 75 min exposure of *E. coli* to UVA-LED as stable current (37.8 mW/cm^2, 0.5 A, and total energy of 15.3 W/cm^2) or pulsed current (3.3 mW/cm^2, 1.0 A, and total energy of 1.3 W/cm^2) inactivated over 99.9 % of *E. coli* cells in aerosols. The curves were biphasic, indicating that UVA-LED irradiation is bactericidal in moving aerosols. A similar biphasic curve was seen after UVC irradiation (10μW/cm^2 and total energy of 87mW/cm^2) at 10 min exposure (Fig.6). The RH during UVA-LED or UVC exposures was around 80% (Fig.5). These results are consistent with those of previous UVGI studies indicating that decay curves are delayed when bacteria

are exposed to relatively low levels of UV irradiation [15]. Furthermore, the high RH used in this study might have led to increased bacterial resistance to UV irradiation; others have reported that airborne bacteria are ten times more resistant to radiation at high *vs.* low relative humidities [16]. This study is a benchmark in research on use of UVA-LED to inactivate bacteria in bioaerosols. It is preliminary, however, since various parameters, such as UVGI bactericidal activity in air, relative RH, UV intensity, and effective bioaerosol sampling require further examination in order to allow firm conclusions. In a second experiment, modified with continuous streams of bioaerosol (data not shown), over 95% of *S. marcescens* or *M. luteus* cells were inactivated by 10 min exposure to constant UVA-LED stable current (0.5 A, 62 mW/cm^2 and a total utilized energy of 37.4 W/cm^2) or pulsed current (1.0 A, 12 mW/cm^2 and total utilized energy of 7.3 W/cm^2). This result shows that the new UVA-LED system has enhanced activity against airborne bacteria in addition to *E. coli*. In this study, conditions of RH, UVA intensity, and bioaerosol sampling were better controlled; these factors might have caused the higher microbial killing ability of the UVA-LED air disinfection system. Our data indicated that bacterial resistance to UVA-LED irradiation can increase with increasing RH (over 50%), which may indicate that UVA-LED air disinfection depends on RH (data not shown). These results are consistent with those previously reported for *S. marcescens* [14], [17] and [18], which indicated significant increases in bacterial survival at RH levels over 50% under UV irradiation. In another experiment, survival of *S. marcescens* decreased with increasing UV dose from UVA-LED stable current, which indicates that the UVA-LED intensity and dose used were above the threshold level for self repair of *S. marcescens* (data not shown); similar results were reported previously [14], [17]. Our results showed that *M. luteus* was more vulnerable to UVA-LED irradiation than *S. marcescens*. This finding is promising regarding the well-known hypothesis that vegetative forms of *M. luteus* are the most resistant to UVGI disinfection [19], [20]. In two successive experiments, similar doses of UVA stable and pulsed current resulted in strikingly different bacterial inactivation rates in which UVA-LED pulsed doses showed approximately five times higher bacterial inactivation than did UVA-LED stable current. This might be related to the higher (2 fold) energy output of UVA-LED pulsed current compared to stable current. Therefore, we suggest that UVA-LED pulsed current is more applicable to practical air disinfection than is stable current; pulsed current has higher microbial killing ability and lower energy consumption. Further studies are needed to establish the germicidal activity of UV-LED over a wide array of airborne contaminants and its possible integration with other air cleaning techniques. In conclusion, we suggest that UVA-LED air disinfection and sterilization has advantages over either LED or UVGI technology used alone.

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