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The New Method for Bacterial Sterilization by Using UVA1 Range Light Emitting Diode

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Abstract

Sterilization using UV-C light has the potential to damage pathogenic bacteria and virus. However, it also has disadvantages such as material deterioration, cellular destruction and limited use depending on the sterilization target. In this study, we focus on the UVA1 light, which is near visible light, but has the potential to induce apoptosis in the cancer cells. To investigate the sterilization characteristics of UVA1 light, three peak wavelengths of 365, 385 and 405 nm were applied by UVA1-LED on *E. coli* colonies with irradiation doses of 0 (control), 10, 50 and 100 J/cm$_2$. Comparing the three peak wavelengths, 365 nm showed a significant effect with an irradiation dose of 100 J/cm^2 after which the colony growth was greatly reduced. These results show that a peak wavelength of 365 nm is sufficient and effective for sterilization if applied with a high power light source, possibly achieving the same effect as sterilization with UV-C light.

Keywords: Bacterial sterilization; *Escherichia coli*; Light Emitting Diode (LED); UVA1 light range

Introduction

Environmental pollution is increasing day by day due to many factors, such as global population growth and inadequate waste disposal [1-3]. As a result, pathogenic bacteria and viruses that did not appear in the past are now being generated [4-7]. Recently, infections due to the pathogenic O157 strain of the *E. coli* bacteria and the avian influenza virus have killed both humans and domestic animals [8-11]. Pollution and unsanitary environments are likely contributors to the significant damage seen among humans and domestic animals. In order to prevent the spreading of such bacteria and viruses, sodium hypochlorite treatments, heat sterilization, ethanol sterilization, UV light (254 nm) sterilization and other methods have been used [12-16]. Each sterilization method is effective, and the UV light with the wavelength of 254 nm is of recognized effectiveness. However, there are some disadvantages in this method such as the deterioration of material and cellular destruction [17,18]. A new sterilization method is therefore required: one which would not damage materials and cells, but would maintain a high sterilization effect. In this study, we focus on the UVA1 light range to investigate the sterilization effect by using ultraviolet light emitting diode (UV-LED) with peak wavelengths of 365, 385 and 405 nm [19].

Materials and Methods

Development of irradiation system by using high-power UVA1-LED

A UVA1 light irradiation system using UVA1-LED was developed with three different peak wavelengths: 365, 385 and 405 nm. The light source in each wavelength was composed of 10 elements of high-power UVA1-LED (LED Engin, Inc.) with the following specifications: λ: 365 nm, FWHM: 12 nm; LZ1-10UV00-0000, λ: 385 nm, FWHM: 13 nm; LZ1-10UA00-U4, λ: 405 nm: FWHM: 17 nm; LZ1-10UA00-U7. No optical lens or reflective plates were used. To obtain high intensity UVA1 light irradiation, the UVA1- LED was lined with the pitch of 1 cm and operated by continuous wave (CW) drive circuit. The light source was cooled by a combination of heat sink and cooling fan. The irradiation distance was manually adjustable from 0 cm to 30 cm.

Evaluation of irradiation intensity characteristics

To evaluate the characteristics of the UVA1-LED irradiation system, the irradiation intensity of the light source was measured. The light source was secured above the center of a power-meter (PM100USB, S310C, Thorlabs, Inc.). The height of the light source varied from 10 cm to 15 cm and the mean irradiation intensity was measured at 1 cm intervals. Figure 1 shows the results of the irradiation intensity. In the light source, optical lens or reflective plates were not used in order to minimize decreased irradiation intensity with increasing irradiation distance.

Evaluation of LB agar mediums temperature characteristics

To investigate the effects of UVA1 light on the bacteria, it is necessary to know the temperature characteristics of the LB agar medium (Difco LB Agar, MILLER (Luria-Bertani), 244520, Becton Dickinson Co. Ltd.) used in this study. Petri dishes with diameters of 6 cm were injected with 5 mL of LB agar medium (solidified) and placed under the UVA1 light source. A thermoelectric couple (TT-533, TANITA Corp.) was pierced into the center of the petri dish. LB agar medium was irradiated with the peak wavelengths of 365, 385 and 405 nm. The height of the light source varied from 10 cm to 15 cm at 1 cm intervals. Temperature was measured at each distance, for 10 minutes at 30 seconds intervals. The experiment was done within an ambient temperature average of 17.3°C and 37% relative humidity. Figure 2 shows the results of these temperature measurements. The LB agar medium temperature

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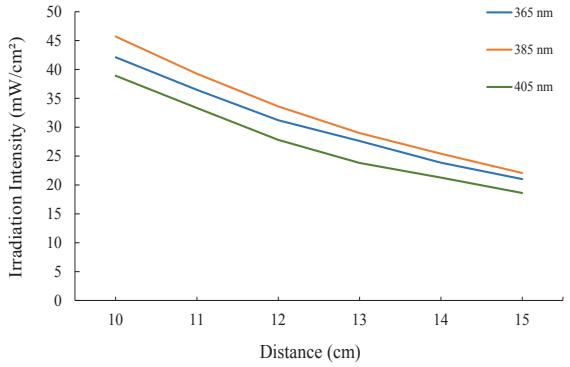

Figure 1: Irradiation intensity measurements. Irradiation intensity of each light source was measured by securing the light source above the center of a power-meter, and taking measurements at different distances, from 10 cm to 15 cm, at 1 cm intervals.

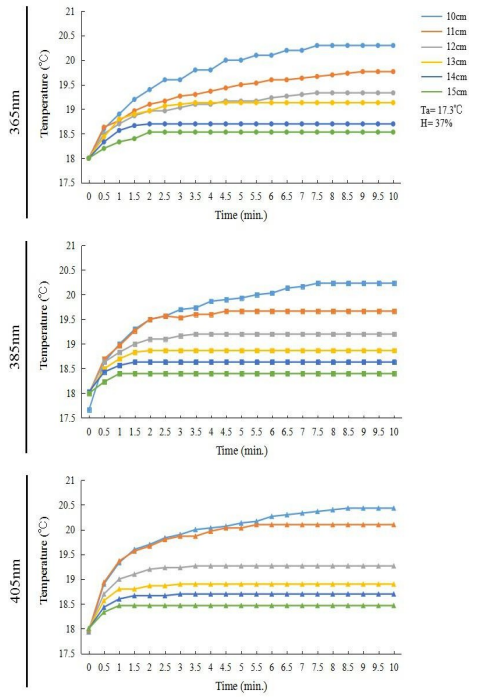

Figure 2: Temperature characteristics of the LB agar medium irradiated by peak wavelengths of 365, 385 and 405 nm (ambient temperature 17.3°C, relative humidity: 37%).

characteristics were similar for wavelengths. At an irradiation distance of 10 cm, temperature increased 2°C in 7 minutes and then stabilized. These results suggest that temperature variations are unlikely to affect the bacteria in this study.

Cultivation and preparation of the *Escherichia coli*

LB agar medium is necessary for the cultivation of *Escherichia coli* (*E. coli*) obtained from National BioResource Project (NIG, Japan: *E. Coli* K12). LB agar medium was prepared as follows: 40 g of powdered Difco LB Agar, MILLER (Difco LB Agar, MILLER (Luria-Bertani), 244520, Becton Dickinson Co. Ltd.) were dissolved in 1 L of purified water and mixed thoroughly. PL agar medium was heated with frequent agitation and boiled for 1 min to completely dissolve the powder, and autoclave at 121°C for 15 min. Finally, 5 mL of PL agar medium were added to the petri dish of 6 cm diameter and solidification was obtained. *E. coli* was cultivated using LB Broth (Miller) Liquid microbial growth medium (L2542-500 mL, Sigma-Aldrich Co. LLC.) in an ambient temperature of 37°C for 12 hours within a shaking-incubator at 122 rpm. After cultivation, *E. coli* concentration was 2×10^6/mL. 10 µL was pipetted off and added to LB agar medium in four places within a distance of 14 mm and cultivated in an ambient temperature of 37°C for 12 hours. Figure 3 shows the *E. coli* colonies prepared for UVA1 light irradiation.

Evaluation of the effect of UVA1 light on *E. coli*

To investigate the effect of UVA1 light on *E. coli*, the peak wavelengths of 365, 385 and 405 nm were applied. Irradiation dose of 0 (control), 10, 50 and 100 J/cm^2 were applied to a petri dish. Each irradiation dose was prepared in one petri dish which contains four *E. coli* colonies as shown in the previous section (Figure 3). Before the UVA1 light irradiation, each *E. coli* colonies size was measured with digital microscopy (Digi Scope, CHRONOS Co.) in order to analyze the difference before and after UVA1 light irradiation. The irradiation experiment was conducted in the following method; the UVA1 light source was fixed at a height where it can obtain 30 mW/cm^2 of irradiation intensity. After irradiation, *E. coli* were cultivated at 37°C for 24 hours. Finally, the size of *E. coli* colonies of each condition was measured with digital microscopy.

Experimental result

Figures 4 and 5 shows the results of *E. coli's* irradiate for UVA1 light with peak wavelengths of 365, 385 and 405 nm. In comparison with control (0 J/cm^2), the colony size of *E. coli* was decreased in proportion to the increase of the irradiation dose. This phenomenon was confirmed at each peak wavelength. However, in comparison with each peak wavelength, it can be judged that the peak wavelength 385 nm and 405 nm are less effective against *E. coli*. On the other hand, 365 nm showed a clear effect. At irradiation dose of 100 J/cm^2, colony appears to growth hardly. In comparison with control, 365 nm for 100 J/cm^2 show a significant difference. These results suggested that sterilization at 365 nm for 100 J/cm^2 or more could be effective to apply in sterilization (** Dunnet test, $p<0.01$, n=4).

Discussion and Conclusion

There are some methods of sterilization for the prevention of

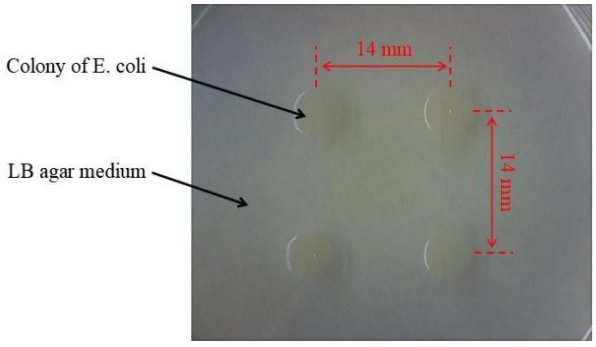

Figure 3: *E. coli* colonies, cultivated in 37°C for 12 hours.

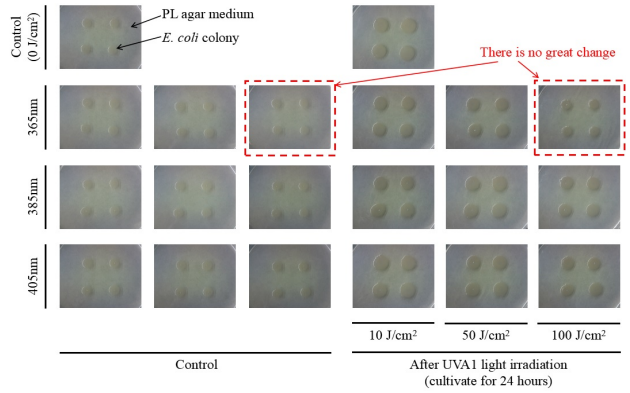

Figure 4: Photograph of each *E. coli* colony before and after the UVA1 light irradiation.

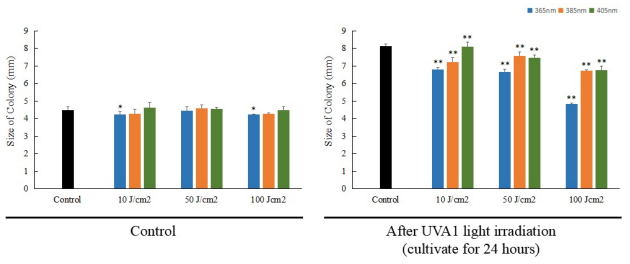

Figure 5: Result of the UVA1 light irradiation experiment. Bars indicate SD. *indicate significant differences (Dunnet test, p<0.05, n=4), **indicate significant differences (Dunnet test, p<0.01, n=4).

pathogenic bacteria and virus such as sodium hypochlorite treatment, heat sterilization, ethanol sterilization, UV-C light sterilization and others. The UV-C light sterilization method has a potential of contact-less sterilization and has high effect. Beck et al. reported the effective characteristic of sterilization using dual-wavelength UV-C LED [16]. All these method can provide a high sterilization effect; however, there have disadvantages of material deterioration, cellular destruction and limit used depending the sterilization target. Therefore, it required a new sterilization method, which not give damage to the materials and cells but could sterilize with high effect. In this study, we focus on the UVA1 light that is near of visible light but have the potential of apoptosis induction on the cancer cells [19]. Three type of UVA1-LED with the peak wavelength of 365, 385 and 405 nm was used for irradiate *E. coli*, and detect the effective peak wavelength which have sterilization potential. Irradiation dose of 0 (control), 10, 50 and 100 J/cm^2 was applied to the *E. coli's* colony. The colony size was measured before the UVA1 light irradiation and after the UVA1 light irradiation cultivated 24 hours. In comparison of the three-peak wavelength, 365 nm showed high effects at irradiation dose 100 J/cm^2 as the colony size almost not grow. On the other hand, peak wavelength 385 nm and 405 nm shows no sterilization effect. From this experimental result, we found that the peak wavelength 365 nm is sufficient and effective for sterilization if apply high power light source. These results suggested the possibility to obtain a same effect of UV-C light.

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