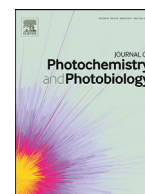




Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Efficacy of 265-nm ultraviolet light in inactivating infectious SARS-CoV-2

Hiroshi Shimoda^a, Junji Matsuda^b, Tatsuyuki Iwasaki^b, Daisuke Hayasaka^{a,*}

^a Laboratory of Veterinary Microbiology, Joint Faculty of Veterinary Medicine, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515, Japan

^b Stanley Electric Co., Ltd., 2-9-13 Nakameguro, Meguro-ku, Tokyo 153-8636, Japan

ARTICLE INFO

Keywords:

265-nm UV
Sars-cov-2
Inactivation, DUV-LED

ABSTRACT

Although, Low-pressure (LP) mercury lamps that emit wavelengths of around 254 nm have been widely applied as ultraviolet (UV) light devices for decontamination of microorganisms, they have raised environmental concerns due to their mercury content. Therefore, UV-LED lamps have high potential for practical use as a replacement for LP mercury lamps. In this study, we evaluated the efficacy of 265-nm UV irradiation in comparison to 254-nm and 280-nm UV irradiation for inactivating infectious severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Irradiation from a 265-nm deep UV light-emitting diode (DUV-LED) lamp efficiently inactivated SARS-CoV-2 at a similar level as a 254-nm UV cold cathode lamp, and at a higher level than a 280-nm DUV-LED lamp.

Introduction

The worldwide spread of novel coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused considerable public health and economic concerns throughout the world [1,2]. Infectious virus particles of SARS-CoV-2 are detected in respiratory droplets from COVID-19 patients, and inactivation of the virus on contaminated surfaces is important for preventing the spread of SARS-CoV-2 infections. Alcohol and other disinfectants are generally used for the disinfection of contaminated materials, but additional measures, such as the use of ultraviolet (UV) light devices, are also useful.

Short-wavelength UV light between 200 and 280 nm (UVC) effectively disinfects viruses, including SARS-CoV-2, because wavelengths in that range with a peak of 260 nm are readily absorbed by nucleic acids, and cause damage to the DNA and RNA of microorganisms. Recently, Heilingloh determined the susceptibility of SARS-CoV-2 to 254-nm UVC and 365-nm UVA irradiation [3], Kitagawa et al. and Buonanno et al. showed the efficacy of 222-nm far-UVC irradiation [4,5], and Inagaki et al. demonstrated the effect of 280-nm deep-UV (DUV) light-emitting diode (LED) irradiation in inactivating SARS-CoV-2⁶.

Low-pressure (LP) mercury lamps that emit wavelengths of around 254 nm have been widely applied as UV light devices due to their low cost and high efficiency. However, they have raised environmental concerns due to their mercury content. Therefore, UV-LED lamps have high potential for practical use as a replacement for LP mercury lamps. Although UV-LED lamps can emit a selectable wavelength between 255 nm

and 280 nm, 265 nm is a practical wavelength to use since it is difficult to regulate shorter wavelengths in UV-LED devices.

In this study, we evaluated the efficacy of 265-nm DUV-LED irradiation in inactivating SARS-CoV-2 in comparison to 254-nm cold cathode lamp (CCL) and 280-nm DUV-LED irradiation. Our results will provide useful information for the development of UV devices for the inactivation of SARS-CoV-2 at various sites, such as hospitals and biosafety laboratories.

Material and methods

African green monkey-derived cells expressing human TMPRSS2 (VeroE6/TMPRSS2 cells; JCRB number: JCRB 1819) were kindly provided by the National Institute of Infectious Diseases (NIID; Tokyo, Japan). The VeroE6/TMPRSS2 cells were propagated in Dulbecco's modified Eagle's minimum essential medium (DMEM; Thermo Fisher Scientific Inc., Waltham, Massachusetts) with 10% heat-inactivated fetal calf serum (JR Scientific, Woodland, CA, USA), 100 U/ml penicillin, and 100 µg/ml streptomycin (Life Technologies, Carlsbad, CA, USA) at 37 °C in 5% CO₂.

SARS-CoV-2 (2019-nCoV/Japan/AI/I-004/2020 strain) was kindly provided by the NIID, and propagated in VeroE6/TMPRSS2 cells grown at 37 °C in 5% CO₂ in DMEM supplemented with 2% fetal calf serum. The supernatants of these cultures were stored at -80 °C as stock viruses.

UV light at 254 nm, 265 nm, and 280 nm was irradiated from a 254-nm UV-CCL, 265-nm DUV-LED, and 280-nm DUV-LED (Stanley Electric Co., Ltd., Tokyo, Japan) device, respectively. The peak wavelength of 265 nm and 280 nm devices were 266 nm and 280 nm, respectively. Although, the peak length of LED can be shifted by high current or tem-

* Corresponding author.

E-mail addresses: hshimoda@yamaguchi-u.ac.jp (H. Shimoda), JUNJI_MATSUDA@stanley.co.jp (J. Matsuda), TATSUYUKI_IWASAKI@stanley.co.jp (T. Iwasaki), dhaya@yamaguchi-u.ac.jp (D. Hayasaka).

<https://doi.org/10.1016/j.jpap.2021.100050>

Received 24 February 2021; Received in revised form 1 June 2021; Accepted 14 June 2021

Available online 17 June 2021

2666-4690/© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

Table 1
Virus titers of SARS-CoV-2 after UV irradiation.

265 nm (DUV-LED)	UV intensity (mW/cm ²)	0.212				
	Irradiated time (s)	0**	6	12	18	24
	UV dose (mJ/cm ²)	–	1.3	2.5	3.8	5.1
	Virus titer (pfu/ml) *	10 ^{5.70} (±0.94)	10 ^{4.78} (±0.77)	10 ^{3.91} (±1.17)	10 ^{3.11} (±0.25)	10 ^{2.49} (±1.68)
254 nm (UV-CCL)	UV intensity (mW/cm ²)	0.357				
	Irradiated time (s)	0**	4	8	11	14
	UV dose (mJ/cm ²)	–	1.4	2.9	3.9	5.0
	Virus titer (pfu/ml) *	10 ^{5.70} (±0.94)	10 ^{4.68} (±0.77)	10 ^{3.64} (±1.02)	10 ^{2.88} (±0.90)	10 ^{2.43} (±1.38)
280 nm (DUV-LED)	UV intensity (mW/cm ²)	0.167				
	Irradiated time (s)	0**	15	30	45	60
	UV dose (mJ/cm ²)	–	2.5	5.0	7.5	10.0
	Virus titer (pfu/ml) *	10 ^{5.70} (±0.94)	10 ^{4.71} (±0.62)	10 ^{3.77} (±0.71)	10 ^{2.88} (±0.74)	10 ^{2.16} (±1.49)

* Geometric mean value (95% confidence interval).

** Control samples (0 s) were not irradiated with any UV light, and the titers were used for comparisons with the irradiated samples.

perature fluctuations of junction, we have installed heatsink and fan more than necessary as their countermeasures. Ten milliliters of 10-fold diluted virus stocks in phosphate-buffered saline were applied to a 50-mm (inner-diameter) dish (Sumitomo Bakelite Inc., Tokyo, Japan) and irradiated for 4, 8, 11, or 14 s (254 nm), 6, 12, 18, or 24 s (265 nm), or 15, 30, 45 or 60 s (280 nm). To make the UV dose to be homogenous within the dish, we have installed collimated tube between the light source and the dish, and the virus solution was stirred by stirrer. Also, since virus solution shows positive absorbance against UV irradiation, the UV intensity (mW/cm²) on the surface of the virus solutions was measured by a chemical actinometer (KI/KIO₃) [7], and calculated using the Lambert-Beer formula [8] to the first place of decimals to prevent the measurement error. All experiments were conducted in triplicate.

VeroE6/TMPRSS2 cells were seeded in 12-well plates with DMEM, and incubated at 37 °C for 24 h. Viruses were 10-fold serially diluted, and 200 µl of each diluted virus was inoculated onto cells. After 90 min at 37 °C, cells were washed twice with DMEM and overlaid with 0.8% agarose (Seaplaque GTG Agarose, Lonza, Rockland, ME, USA) in DMEM. After 1.5 days of incubation, the cells were fixed with 10% buffered formaldehyde, and the agarose layer was removed. After staining with crystal violet, plaques were counted to calculate the virus titer.

Results

The infectious viral titers were significantly reduced by 265-nm UV irradiation (non-irradiated control: 10^{5.70} pfu/ml (95% confidence interval: ±0.94 pfu/ml); 24 s of irradiation: 10^{2.49} pfu/ml (95% confidence interval: ±1.68 pfu/ml) (Table 1). The 254-nm UV irradiation also rapidly inactivated the infectious virus; it reduced the viral titer to 10^{2.43±1.38} pfu/ml after 14 s (Table 1). The 280-nm UV irradiation also caused a time-dependent reduction of virus titers, but the efficiency was lower than those of the 265-nm and 254-nm UV irradiation (Table 1).

Based on these data, we calculated the UV doses (mJ/cm²) for the inactivation of SARS-CoV-2 (Fig. 1). The UV doses for 3-log reductions of the virus titers were 4.5, 4.4, and 8.2 mJ/cm² following 265-nm DUV-LED, 254-nm UV-CCL, and 280-nm DUV-LED irradiation, respectively. These data indicated that 265-nm DUV-LED irradiation caused similar levels of SARS-CoV-2 inactivation as 254-nm UV-CCL irradiation, whereas 280-nm DUV-LED irradiation was less efficient than the 265-nm and 254-nm UV irradiation.

Discussion

In this study, we showed that 265-nm DUV-LED irradiation efficiently inactivated SARS-CoV-2 at a similar level as 254-nm UV-CCL irradiation. In the previous study by Inagaki et al., 280-nm DUV-LED irradiation resulted in more than a 3-log reduction in the SARS-CoV-2 titer within 10 s; however, they did not show the UV doses for virus reduction [6]. Here, we calculated the UV doses for 3-log reductions,

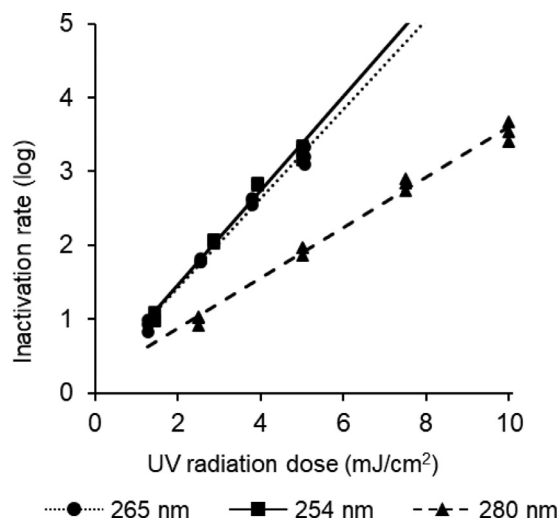


Fig. 1. Inactivation rates of SARS-CoV-2 by UV light. The X-axis indicates the UV radiation dose, and the Y-axis indicates the inactivation rate of the log phase. Each line indicates the approximation line calculated from the results for each wavelength.

and showed that irradiation at 265 nm and 254 nm were more efficient than that at 280 nm.

In the previous study by Inagaki et al. [6] indicated that approximately 3 log reductions of SARS-CoV-2 by 280 nm UVC-LED irradiation with UV dose of 37.5mJ/cm², which was 10mJ/cm² in our study. Also, Heilingloh et al. [3] indicated that 254 nm UVC-LED irradiation with UV dose of 350mJ/cm² caused 3 log reduction of SARS-CoV-2, while only UV dose of 5mJ/cm² were necessary in this study. These differences may be due to the difference of experimental methods that smaller quantity of virus solution was used in the previous study compared to our study. It may be necessary to standardize the experimental methods for comparative analysis of UV irradiation. Heilingloh et al. [3] also provided the data on irradiation of UV-A (365 nm) against SARS-CoV-2, which resulted in less than 2 log reductions even after the irradiation with UV dose of 500mJ/cm². Therefore, irradiation of UV-C region rather than UV-A is necessary for rapid disinfection of SARS-CoV-2.

UV inactivation systems are useful for the disinfection of pathogenic organisms, including viruses. Germicidal lamps emitting UVC at around 254 nm are commonly applied in UV disinfection systems, but they have raised environmental concerns due to their mercury content. As a replacement for germicidal lamps, LED lamps have been widely applied for UV systems. Although excessive UV-C (100–280 nm) radiation exposure has the potential to cause harmful effects to the eye skin and immune system, the 265-nm DUV-LED lamp used in this study is a small

light source that can be built into any kind of device, such as medical equipment and devices in food factory lines, etc. as implemented previously [9,10]. These UV-equipped devices are expected to prevent not only SARS-CoV-2 transmission and infection, but also those by other viruses and bacteria. Further examinations to assess the efficacy of 265-nm DUV-LED irradiation in inactivating other pathogens and also when contaminations occurs in materials with different characterization will provide useful information for the control of pathogenic microorganisms, including viruses.

Acknowledgements

We thank NIID for providing SARS-CoV-2 (2019-nCoV/Japan/AI/1-004/2020 strain) and VeroE6/TMPRSS2 cells (JCRB number; JCRB 1819). This work was supported financially by Stanley Electric Co., Ltd., JSPS KAKENHI Grant Numbers JP17H04661, and MHLW Program Grant Number JPMH20HA2010.

References

- [1] Q. Li, X. Guan, P. Wu, et al., Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia, *N. Engl. J. Med.* 382 (2020) 1199–1207.
- [2] P. Skums, A. Kirpich, P. Icer Baykal, et al., Global transmission network of SARS-CoV-2: from outbreak to pandemic., *medRxiv* (2020).
- [3] C.S. Heilingloh, U.W. Aufderhorst, L. Schipper, et al., Susceptibility of SARS-CoV-2 to UV irradiation, *Am. J. Infect. Control* 48 (2020) 1273–1275.
- [4] M. Buonanno, D. Welch, I. Shuryak, et al., Far-UVC light (222 nm) efficiently and safely inactivates airborne human coronaviruses, *Sci. Rep.* 10 (2020) 10285.
- [5] H. Kitagawa, T. Nomura, T. Nazmul, et al., Effectiveness of 222-nm ultraviolet light on disinfecting SARS-CoV-2 surface contamination, *Am. J. Infect. Control* (2020).
- [6] H. Inagaki, A. Saito, H. Sugiyama, et al., Rapid inactivation of SARS-CoV-2 with deep-UV LED irradiation, *Emerg. Microbes Infect.* 9 (2020) 1744–1747.
- [7] R.O. Rahn, M.I. Stefan, J.R. Bolton, et al., Quantum yield of the iodide-iodate chemical actinometer: dependence on wavelength and concentrations, *Photochem. Photobiol.* 78 (2003) 146–152.
- [8] J.R. Bolton, K.G. Linden, Standardization of Methods for Fluence (UV Dose) Determination in Bench-Scale UV Experiments, *J. Environ. Eng.* 129 (3) (2003) 209–215.
- [9] G. Messina, S. Burgassi, D. Messina, et al., A new UV-LED device for automatic disinfection of stethoscope membranes, *Am. J. Infect. Control* 43 (10) (2015) e61–e66.
- [10] A. Prasad, L. Du, M. Zubair, et al., Applications of Light-Emitting Diodes (LEDs) in food processing and water treatment, *Food Eng. Rev.* 12 (2020) 268–269.