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Comparison of the inactivation capacity of various UV wavelengths on SARS-CoV-2

Ryosuke Matsuura a,b, Chieh-Wen Lo a,b,c, Takayo Ogawa d, Masaru Nakagawa b, Masami Takei b, Yasunobu Matsumoto a,c, Satoshi Wada b,d, Yoko Aida a,b,c,d,*

a Laboratory of Global Infectious Diseases Control Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo, 113-8657, Japan
b Division of Hematology and Rheumatology, Department of Medicine, Nihon University School of Medicine, 30-1 Oyaguchi-kamiuschou, Itabashi-ku, Tokyo, 173-8610, Japan
c Laboratory of Global Animal Resource Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo, 113-8657, Japan
d Photonics Control Technology Team, RIKEN Center for Advanced Photonics, Hirosawa 2-1, Wako, 351-0198, Japan

* Corresponding author. Laboratory of Global Infectious Diseases Control Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo, 113-8657, Japan.
E-mail address: yoko-aida@g.ecc.u-tokyo.ac.jp (Y. Aida).

ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused a worldwide pandemic. Ultraviolet (UV) is regarded as a very powerful tool against SARS-CoV-2. However, the inactivating effects of different UV wavelengths on SARS-CoV-2 under the same conditions have hardly been compared. Here, we showed that SARS-CoV-2 cultured in Dulbecco’s modified Eagle’s medium and 2% fetal bovine serum was efficiently inactivated by irradiation with 222, 254, and 265 wavelengths UV, but not at 308 nm. In addition, it was revealed that UV absorption by DMEM-2% FBS is very efficient at 222 nm. Our results present potentially important information for selecting the optimum UV wavelength according to the application.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19) and has led to a pandemic [1]. In addition, SARS-CoV-2 has had an unprecedented impact on modern human civilization, and resulted in more than 6.25 million deaths globally as of May 2022. The number of infected and dead individuals is still increasing despite the development of vaccines and drugs. SARS-CoV-2 is transmitted through air, surface contamination, and fecal-oral contamination [2]. Thus, inactivating SARS-CoV-2 in the environment is essential for controlling its transmission. Indeed, it was reported that SARS-CoV-2 is inactivated by photocatalysts [3], heat [4], ultraviolet (UV) irradiation [5,6], and disinfectants such as ethanol [7].

UV is one of the well-researched tools, as it is known that UV at various wavelengths has a virus inactivating effect. Based on wavelengths, UV is classified as UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm) [8]. Low pressure mercury lamps with a UV wavelength of 254 nm are usually used for sterilization and to rapidly reduce SARS-CoV-2 viability [5,6]. In addition, it is reported that UVC at 254 nm inactivates SARS-CoV-2 through the induction of viral genome damage [6]. Recent improvements in optical technology have made it possible to manufacture light-emitting diodes (LEDs) with UV of various wavelengths, and specially, wavelengths at 222 [9–11], 250 [12], 254 [5,6,9,13], 257 [14], 265 [13,15], 270 [9], 280 [12,13,15], 282 [9], and 300 nm [15], inactivate SARS-CoV-2. Particularly, UVC at 222 nm wavelength has been actively researched because of its inactivation ability and harmlessness [9–11]. It has been reported that not only UVC but also UVB, UVA, and sunlight can inactivate SARS-CoV-2 [5,15,16]. However, the virus inactivation effect of UVA and UVB is weaker than that of UVC [5,15], as the virus inactivation effect of UV differs depending on the wavelengths. In addition, the virus inactivation ability of UV is affected by impurities such as fetal bovine serum (FBS) and salt [15]. Therefore, it is very important to investigate the virus inactivation ability at various wavelengths of UV under the same experimental conditions. However, there are not many studies comparing the inactivating effect of UV of various wavelengths on SARS-CoV-2.

In this study, we selected four kinds of light sources of UV. One was 222 nm LED which was the only light source with a wavelength of below

https://doi.org/10.1016/j.bbrep.2022.101379
Received 25 August 2022; Received in revised form 5 October 2022; Accepted 3 November 2022
Available online 7 November 2022
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230 nm. A low pressure mercury lamp at 254 nm was also used because it is the most popular, cheap and easily available UV light source. A 265 nm LED that has the shortest wavelength with high-power LED was also studied. Furthermore, a 308 nm LED was selected as UVB, as inactivation of SARS-CoV-2 by this wavelength has never been reported.

2. Material and methods

2.1. Cell and virus

Vero E6/TMPRSS2 cells (Japanese Collection of Research Bioresources no. JCRB1819) were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO, USA), 1% penicillin/streptomycin/glutamine (PSG), and 2% G418 (Thermo Fisher Scientific) at 37°C with 5% CO₂.

SARS-CoV-2 (SARS-CoV-2/JPN/TY/WK-521 strain) received from the National Institute of Infectious Diseases of Japan [17] was propagated using Vero E6/TMPRSS2 cells cultured in DMEM containing 2% FBS and 1% PSG.

2.2. UV irradiation to SARS-CoV-2

SARS-CoV-2 at a concentration of 5 × 10⁴ TCID₅₀/mL in DMEM containing 2% FBS was irradiated by a UV at 222, 254, 265, and 308 nm wavelengths for 0, 5, 15 or 30 mJ/cm². Subsequently, the titer of SARS-CoV-2 was measured by the TCID₅₀ assay with Vero E6/TMPRSS2 cells (JCRB no. JCRB1819), as previously described [3,6].

2.3. SARS-CoV-2 titration by 50% tissue culture infectious dose (TCID₅₀) assay

Vero E6/TMPRSS2 cells cultured in a 96-well plate (2 × 10⁴ cells per well) were infected with 100 μL of 10-fold serially diluted virus-containing infection medium (each dilution had 8 replicates) and incubated at 37°C for 3 days. Following incubation, viral infection in each well was determined based on the virus-induced cell cytopathic effect and infectivity was then calculated using the Reed-Muench method [18].

2.4. The optical absorbance

Optical absorbance of 2% FBS in MilliQ and DMEM containing 2% FBS at 222, 254, 265 and 308 nm wavelengths was measured using NanoDrop One (Thermo Fisher Scientific).

2.5. Statistical analysis

Two-way analysis of variance (ANOVA) with Dunnett’s test was used to compare all samples with the sample obtained at 0 mJ/cm² for statistical determination. ANOVA followed by Tukey’s test was performed to compare each wavelength and each optical density. p values < 0.05 were considered statistically significant. All calculations were performed using R software (version 3.6.3, R Foundation for Statistical Computing, Vienna, Austria).
3. Result and discussion

3.1. Inactivation of SARS-CoV-2 by UV at various wavelength

To determine the UV inactivation ability of SARS-CoV-2 at various wavelengths, SARS-CoV-2 in DMEM containing 2% FBS was irradiated by a UV at each wavelength for 0, 5, 15, or 30 mJ/cm² (Fig. 1A). As shown in Fig. 1B, virus infectivity after UV irradiation at 222, 254, and 265 nm significantly decreased depending upon the light intensity for 5, 15, and 30 mJ/cm². By contrast, no reduction in viral titer was observed at irradiation with UV at 308 nm. Interestingly, 254 nm UV was the most effective among all wavelengths under consideration. Although some studies have reported that 222 nm UV can efficiently inactivate SARS-CoV-2 [9–11], 265 nm UV showed a higher virus inactivation effect (Fig. 1C).

As mentioned above, in this study, the strength of the virus inactivation effect of UV was in the order of 254, 265, 222 nm wavelengths, but no virus inactivation was observed at 308 nm. However, in previous studies, 222 nm showed a higher inactivation effect than 254 nm [9]. Other studies have shown that there is little difference between 254 and 265 nm [13]. In particular, in a previous study of SARS-CoV-2 inactivation by 222 nm UV, the titers of SARS-CoV-2 was found to be decreased by 1.42 log_{10} TCID₅₀/ml at 1 mJ/cm² [9] and 4.4 and 2.51 log_{10} TCID₅₀/ml at 3 mJ/cm² [10,11]. In contrast, in this study, the titers of SARS-CoV-2 were decreased by 1.25 log_{10} TCID₅₀/ml at 30 mJ/cm². Thus, unlike previous studies, the efficiency of 222 nm UV in inactivating the virus was found to be low in this study. However, the conditions of previous studies were different from those of the current study. Ma et al. diluted SARS-CoV-2 in PBS and irradiated it with UV, and Kitagawa et al. applied SARS-CoV-2 in DMEM-10% FBS on polystyrene plates, and irradiated the samples with UV after drying [9–11]. Therefore, it is possible that the UV was not absorbed by DMEM and FBS; it would have inactivated the virus more efficiently, as in the previous study. Indeed, previous studies have reported that MEM and FBS have an absorption spectrum in the UV region, and proteins such as casein, papain, and zein have a very high absorption spectrum at 222 nm [19]. Therefore, under this experimental condition, the compounds and FBS contained in the virus solution absorbed UV and might have reduced the inactivation of the virus.

3.2. The optical absorbance of medium and FBS at various wavelength

To assess the optical absorption characteristics of 2% FBS, and DMEM containing 2% FBS, UV absorbance of 2% FBS in MilliQ and DMEM containing 2% FBS at 222, 254, 265 and 308 nm wavelengths was measured. As shown in Fig. 2A, the absorbance of FBS and DMEM at each wavelength was significantly higher compared with that of MilliQ, which was the blank. Especially, the absorbances of FBS and DMEM at 222 nm were 7.84 and 18.1, respectively, which is considerably high. Indeed, the absorbances of FBS and DMEM at 222 nm were significantly higher compared with those at 254, 265 and 308 nm (Fig. 2B). This result suggests that any protein and chemical compound present in FBS and DMEM absorb the UV, and this absorbance might reduce the inactivation of SARS-CoV-2 at 222 nm UV.

It is believed that 222 nm UV, unlike 254 nm UV, cannot penetrate the epidermis [20]. Therefore, it is considered that there is no risk of 222 nm UV reaching the dermis, damaging the DNA, and leading to the development of cancer [20]. In addition, because 222 nm UV cannot inactivate viruses as in previous studies, it has currently received considerable attention [9–11]. However, the results of this study suggest that 222 nm UV may be affected more by contamination such as proteins than 254 nm UV; therefore, further research is necessary to clarify their effectiveness in the real environment.

3.3. Significance of present research and development of future research

Here, we have reached two major conclusions: First, viability of SARS-CoV-2 significantly decreases with UVC at 222, 254, and 265 nm wavelengths. In particular, it is clear that SARS-CoV-2 in DMEM containing 2% FBS is sensitive to UV in the order of 254, 265, and 222 nm wavelengths. In contrast, UV at 308 nm does not decrease the titer of SARS-CoV-2. This is the first report of a comparison of the SARS-CoV-2 inactivation ability of UV at 222, 254, 265, and 308 wavelengths under the same conditions, where viruses in DMEM contained 2% FBS. Second,
UV at 254, 265, and 308 nm is also absorbed by DMEM-2% FBS, but the absorption rate is significantly lower than that at 222 nm. This could be because the salts and proteins contained in DMEM and FBS absorb UV at 222 nm. This result suggests that the solvent has a very large effect on the inactivation of the virus by UV.

There has been no report on the inactivation of SARS-CoV-2 by UV at 308 nm, but it has been reported that UVB at 300 nm inactivated 99.9% of SARS-CoV-2 in vitro irradiation. Am. J. Infect. Control. Am. J. Infect. Control 48 (2020) 1273.

In addition, UVA at 365 nm has been reported to reduce 1 log<sub>10</sub> TCID<sub>50</sub>/mL at 292 mJ/cm<sup>2</sup> [5]. Furthermore, it has been reported that the 50% effective dose of UV at 300–450 nm, which imitates sunlight, is 62.4 mJ/cm<sup>2</sup> or 54.9 mJ/cm<sup>2</sup> [16]. From these previous reports, it can be suggested that UVA and UVB also have virus inactivation abilities. However, although the antiviral effect of UV at 308 nm could not be confirmed in this study, it is possible that it may show an inactivating effect if the illumination is increased further.

This study shows that UV, especially UVC, is useful for SARS-CoV-2 inactivation, and UV is very important for preventing the spread of SARS-CoV-2 infection. In addition, it suggests the importance of comparing the antiviral effects of UVs of different wavelengths under the same conditions. The characteristics of UV are variable depending upon its wavelength. For example, 222 nm UV is harmless to the human body, whereas 254 and 265 nm UV is effective against viruses with protein contamination, and UVA and UVB are components of sunlight. Therefore, it is very important to select the optimum wavelength of UV according to the application. Accordingly, further research on the effectiveness of UV at various wavelengths under real environmental conditions, such as droplets, is necessary.

4. Conclusion

In this study, to investigate the virus inactivation ability of UV at various wavelengths under the same experimental conditions, SARS-CoV-2 was irradiated with various UV wavelengths under the same conditions. The inactivation ability was found to reduce in the order of 254, 265 and 222 nm UV wavelengths. In contrast, 308 nm UV did not show any inactivation ability. In addition, it was clarified that UV, especially at 222 nm, is strongly absorbed by FBS. These results suggest that protein contamination has a considerable effect on the inactivation of SARS-CoV-2 by UV. In conclusion, this study presented potentially important information for selecting the optimum UV wavelength according to the application.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

We would like to thank Kalttech Co., Ltd. (https://kalteck.co.jp/en/) for helping the organization of our laboratory. We also thank the National Institute of Infectious Diseases of Japan for kindly providing of SARS-CoV-2 (SARS-CoV-2-JPN/TY/WK-521 strain). We are grateful to all members of Laboratory of Global Infectious Diseases Control Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, and Division of Hematology and Rheumatology, Department of Medicine, Nihon University School of Medicine, for their technical assistance, help, and suggestions. This work was supported by a grant from Office for Novel Coronavirus Disease Control, Cabinet Secretariat, Government of Japan (COVID-19 AI and Simulation Project). We would like to thank Editage (www.editage.com) for English language editing.

References

[1] F. Wu, S. Zhao, B. Yu, Y.M. Chen, W. Wang, Z.G. Song, Y. Hu, Z.W. Tao, J.H. Tian, Y.Y. Pei, M.L. Yuan, Y.L. Zhang, F.H. Dai, Y. Liu, Q.M. Wang, J.J. Zheng, L. Xu, F. C. Holmes, Y.Z. Zhang, A new coronavirus associated with human respiratory disease in China, Nature 579 (2020) 265.
[2] R. Mukhra, K. Krishan, T. Kanchan, Possible modes of transmission of Novel coronavirus SARS-CoV-2: a review, Acta Biomed. 91 (2020), e2020036.
[3] R. Matsuura, C.W. Lo, S. Wada, J. Somei, H. Ochiai, T. Murakami, N. Saito, T. Ogawa, A. Shinojo, Y. Benno, M. Nakagawa, M. Takei, Y. Aida, SARS-CoV-2 disinfection of air and surface contamination by TiO<sub>2</sub> photocatalyst-mediated damage to viral morphology, RNA, and protein, Viruses 12 (2020) 650.
[4] F. Saadatpour, F. Mohammadianbah, Physicochemical susceptibility of SARS-CoV-2 to disinfection and physical approach of prophylaxis, Health Sci. Rep 3 (2020), e213.
[5] C.S. Heilingloh, U.W. Außerhorst, L. Schipper, U. Dittmer, O. Witzke, D. Yang, X. Zheng, K. Sutter, M. Trilling, M. Alt, E. Steinmann, A. Krawczyk, Susceptibility of SARS-CoV-2 to UV irradiation. Am. J. Infect. Control. Am. J. Infect. Control 48 (2020) 1273.
[6] C.W. Lo, R. Matsuura, K. Ihimura, S. Wada, A. Shinojo, Y. Benno, M. Nakagawa, M. Takei, Y. Aida, UVC disinfects SARS-CoV-2 by induction of viral genome damage without apparent effects on viral morphology and proteins, Sci. Rep. 11 (2021), 13804.
[7] A. Kratzel, D. Todi, P. Vovkovi, S. Steiner, M. Gultom, T.T.N. Thao, N. Ehret, M. Holwerda, J. Steinmann, D. Niemeyer, R. Dijkman, G. Kampf, C. Drosten, E. Steinmann, V. Thiel, S. Pfänder, Inactivation of severe acute respiratory syndrome coronavirus 2 by WHO recommended hand rub formulations and alcohols, Emerg. Infect. Dis. 26 (2020) 1592.
[8] J.A. Guerrero-Beltr, G.V. Barbona, Advantages and limitations on processing foods by UV light, Food Sci. Technol. Int. 10 (2004) 137.
[9] B. Ma, P.M. Gundy, C.P. Gerba, M.D. Soebe, K.G. Linden, UV inactivation of SARS-CoV-2 across the UVC spectrum: KrCl* excimer, mercury-vapor, and light-emitting diode (LED) sources, Appl. Environ. Microbiol. 87 (2021), e0151221.
[10] H. Kitagawa, T. Nomura, T. Nazmul, K. Kawano, K. Omori, N. Shimogoto, T. Sakaguchi, H. Ohge, Effect of intermittent irradiation and fluence response of 222 nm ultraviolet light on SARS-CoV-2 contamination, Photodiagnostics Photodyn. Ther. 33 (2021), 102184.
[11] H. Kitagawa, T. Nomura, T. Nazmul, K. Omori, N. Shimogoto, T. Sakaguchi, H. Ohge, Effectiveness of 222 nm ultraviolet light on disinfecting SARS-CoV-2 surface contamination, Am. J. Infect. Control 49 (2021) 299–301.
[12] M. Bornmann, M. Alt, L. Schipper, L. van de Sand, M. Otte, T.L. Meister, U. Dittmer, O. Witzke, E. Steinmann, A. Krawczyk, Disinfection of SARS-CoV-2 contaminated surfaces of personal items with UV-LED disinfection boxes, Viruses 13 (2021) 598.
[13] H. Shimoda, J. Matsuoka, T. Iwasaki, D. Hayakawa, Efficacy of 265-nm ultraviolet light in inactivating infectious SARS-CoV-2, J. Photochem. Photobiol. A, 7 (2021), 101050.
[14] S. Liu, W. Luo, D. Li, Y. Yuan, W. Tong, J. Kang, Y. Wang, D. Li, X. Rong, T. Wang, Z. Chen, Y. Li, H. Wang, W. Wang, J. Hoo, L. Yan, S. Guo, B. Shen, Z. Cong, X. Wang. See-eliminating the SARS-CoV-2 by AlGaN based high power deep ultraviolet light source, Adv. Funct. Mater. 25 (2020), 2008452.
[15] T. Minamikawa, T. Koma, A. Suzuki, T. Mizuno, K. Nagamatu, H. Arimochi, K. Tsuchiya, K. Matsuoka, T. Yasui, K. Yanutomo, M. Nomaguchi, Quantitative evaluation of SARS-CoV-2 inactivation using a deep ultraviolet light-emitting diode, Sci. Rep. 11 (2021) 5070.
[16] G.T. Wondrak, J. Jandova, S.J. Williams, D. Schenten, Solar simulated ultraviolet radiation inactivates HCoV-NL63 and SARS-CoV-2 coronaviruses at environmentally relevant doses, J. Photochem. Photobiol. B, 224 (2021), 112319.
[17] S. Matsuura, N. Nao, K. Shirato, M. Kawase, S. Saito, I. Takayama, N. Nagata, T. Sekizuka, H. Katoh, M. Sakata, M. Tahara, S. Kutsuna, N. Ohmagari, M. Kuroda, T. Suzuki, T. Kageyama, M. Takeda, Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells, Proc. Natl. Acad. Sci. USA 117 (2020) 7001.
[18] L.J. Reed, H. Muench, A simple method of estimating fifty per cent endpoints 12, Proc. Natl. Acad. Sci. USA 1938 (1938) 493–497.
[19] R. Stefanescu, S. Brebua, M. Matei, I.M. Risca, A. Surleva, G. Drochioiu, Contribution to casein determination by UV spectrophotometry, Acta Chem. Iasi 25 (2017) 112.
[20] J. Cadet, Harmless effects of sterilizing 222-nm far-UV radiation on mouse skin and eye tissues, Photobiol. Photobiol. 96 (2020) 949.