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SHORT COMMUNICATION

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Rapid inactivation of *Dabie bandavirus* (SFTSV) by irradiation with deep-ultraviolet light-emitting diode

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Abstract

Severe fever with thrombocytopenia syndrome (SFTS) caused by *Dabie bandavirus* (SFTSV) is a serious public health concern in endemic areas, particularly in Asian and Southeast Asian countries. SFTSV is transmitted by direct contact with body fluids from infected humans and animals. Therefore, environmental hygiene in hospitals and veterinary clinics in SFTSV-endemic areas is highly important. This study assessed the effects of continuous and intermittent irradiation with deep-ultraviolet light-emitting diode (DUV-LED) on SFTSV. Evaluation was performed by conducting plaque assay in which SFTSV irradiated with deep-ultraviolet (DUV; 280 ± 5 nm) was inoculated onto Vero cells. The results showed that continuous and intermittent irradiation for 5 s, resulting in 18.75 mJ/cm^2 of cumulative UV exposure, led to a >2.7 and >2.9 log reduction, respectively, corresponding to a >99.8% reduction in infectivity. These results demonstrate that DUV can be utilized for inactivation of SFTSV to maintain environmental hygiene in hospitals and veterinary clinics in endemic countries.

KEYWORDS

Dabie bandavirus (SFTSV), DUV-LED, environmental hygiene, inactivation, severe fever with thrombocytopenia syndrome (SFTS)

1 | INTRODUCTION

Dabie bandavirus, or severe fever with thrombocytopenia syndrome virus (SFTSV), is the causative agent of severe fever with thrombocytopenia syndrome (SFTS). SFTSV, which belongs to the genus *Bandavirus* within the family *Phenuiviridae* (order *Bunyavirales*),[1] is a tick-borne zoonotic virus that infects humans, various domestic animals, and a variety of wild animals.[2–4] The disease was first reported in China,[3] followed by reports in South Korea[5] and other Asian or Southeastern Asian countries, including Japan.[6–8] As of July

2021, 641 human SFTS cases were reported in 26 of 47 prefectures in Japan with high case fatality rate, although most were in western parts of the country.[9] SFTSV is transmitted through direct contact with body fluids from infected humans or animals, even in the absence of tick bites[10,11]; therefore, workers in animal-related occupations such as veterinarians and veterinary assistants are at high risk of infection with SFTSV. Thus, prevention is important in endemic areas. However, the need to be prepared to receive SFTSV-infected animals (i.e., having appropriate personal protective equipment and environmental hygiene), particularly in veterinary clinics, is not fully appreciated, even in endemic countries.[12]

A deep-ultraviolet light-emitting diode (DUV-LED) instrument that generates wavelengths around 250–300 nm has been evaluated for its ability to inactivate microorganisms, including an emergent virus such as severe acute respiratory syndrome coronavirus-2.[13,14] Moreover, reports on the inactivation of microorganisms by emitting intermittent irradiation besides continuous irradiation have increased.[15] Intermittent irradiation is a pattern of DUV generation utilizing the ability of DUV-LED that can be turned on and off with high frequency. The high flexibility of intermittent irradiation, which is offered by the features of DUV-LED with various frequencies and duty rates and low power requirement for operation, brings potential on the application of intermittent DUV-LED irradiation.[15] Here, we examined the ability of continuous and intermittent DUV-LED irradiation to inactivate SFTSV, as well as its possible application to the veterinary field as a public health measure.

2 | MATERIALS AND METHODS

Vero cells cultured in Eagle's minimum essential medium (MEM; Sigma-Aldrich), containing 10% fetal bovine serum (FBS; Biowest), 100 units/ml penicillin and 100 μg/ml streptomycin (FUJIFILM Wako Pure Chemical), and 10 mM HEPES (Nacalai Tesque), were seeded (0.5×10^5 cells per well) into 24-well plates and incubated for 24 h before the assay. The SFTSV strain used in this study was isolated from the tick *Haemaphysalis formosensis*, caught in Miyazaki prefecture (A17/*H. formosensis*/Miyazaki/2016; Accession nos. LC536536.1, LC536546.1, and LC536556.1 for the S, M, and L segment, respectively).[16] The DUV-LED apparatus, "SumiRay-VPS164," which generates a narrow wavelength (280 ± 5 nm), was obtained from Nikkiso Co., Ltd. and is described in a previous study.[14]

The effect of DUV-LED irradiation was evaluated using the method of Inagaki et al.[14] Briefly, aliquots of MEM containing SFTSV were placed in the center of a 60 mm Petri dish (Greiner Bio-One), and a droplet of the medium was set under the DUV-irradiation port of the apparatus (Appendix S1). The distance between the bottom of the Petri dish and the irradiation port was 20 mm, and the duration of irradiation was 1, 5, or 10 s ($n = 3$ for each) (Appendix S1). DUV was delivered as 3.75 mW/cm^2 of continuous irradiation, at a current of 0.35 A, a duty rate of 100%, and a frequency of 0 KHz; or as 7.5 mW/cm^2 of intermittent irradiation, at a current of 0.70 A, a duty rate of 50%, and a frequency of 1 kHz (Appendix S2). Cumulative UV exposure from 1 to 10 s ranged from 3.75 to 37.5 mJ/cm^2 under both continuous and intermittent irradiation conditions.

The above mentioned aliquots of SFTSV irradiated with DUV were serially diluted 10-fold in serum-free MEM in a 96-well plate. Subsequently, the virus diluents were inoculated onto Vero cells seeded in 24-well plates as described above. After adsorption of the inoculum for 1 h at 37°C/5% CO_2, cells were overlaid with MEM containing 1.5% carboxymethyl cellulose and 2% FBS. The cells were then incubated for 8 days at 37°C/5% CO_2. Finally, cells were fixed with 10% formalin for 30 min and stained with 2% crystal violet solution before counting plaques.

The antiviral effects on DUV-LED irradiation were assessed by calculating the log reduction as follows: $\log_{10}(N_0/N_t)$, where N_0 is the number of plaque forming units (PFU) of the sample without UV irradiation, N_t is the number of PFU of the UV-irradiated sample for the irradiation time t. When expressing the results, the number of digits after the second decimal point was truncated. Percent reduction was calculated as follows: $[1 - 10^{-(\log \text{ reduction})}] \times 100$ (%); the mean log reduction was used for the calculation, and the number of digits after the second decimal point was truncated.

Statistical analysis was performed using Dunnett's test to detect significant differences in the infective virus titer after DUV-LED irradiation. The test was conducted using R package "multcomp" (R software version 4.1.1; R[17]). A $p < 0.05$ was considered statistically significant.

3 | RESULTS AND DISCUSSION

The titers of infective SFTSV (PFU/ml) were reduced significantly after both continuous and intermittent irradiation (Table 1 and Figure 1A). DUV-irradiation for 5 s (equivalent to 18.75 mJ/cm^2) reduced SFTSV infectivity by >99.8% (under both continuous and intermittent irradiations), which yielded log reductions of >2.7 and >2.9, respectively (Table 1 and Figure 1B). The present study demonstrates rapid inactivation of SFTSV by DUV-LED irradiation. Intermittent irradiation was as effective as continuous irradiation with the same amount of cumulative UV exposure (mJ/cm^2). Thus, use of intermittent irradiation could be an energy-saving feature of future DUV-LED products.

Veterinarians and veterinary technicians are at high risk of SFTSV infection due to their exposure to infected companion animals, especially cats, which shed high titers of the virus.[10,12] Therefore, treatment and care of SFTSV-infected animals require special precautions. Such animals must be isolated from other animals. Environmental hygiene in clean areas within veterinary clinics should be carefully maintained to avoid virus contamination from the areas in which infected animals are kept. In such situations, rapid, effective, and uncomplicated virus-inactivation methods are necessary to sterilize materials and the environment. Furthermore, sterilization by DUV will make up for the disadvantages of disinfection with irritant and smelly disinfectants for animals. The results presented in this study show that DUV-LED is

TABLE 1 Differences in infectious virus titer after continuous and intermittent deep-ultraviolet light-emitting diode (DUV-LED) irradiation

	Control (without irradiation)	DUV-LED irradiation time (n = 3, each)		
		1 s	5 s	10 s
Continuous irradiation				
Plaque forming units (PFU: PFU/ml)[a]	$9.7\ (\pm 2.8) \times 10^4$	$8.3\ (\pm 2.3) \times 10^3$	$<1.7\ (\pm 0.6) \times 10^2$	$<1.0 \times 10^2$
Log reduction[b]	–	1.0 ± 0.1	>2.7 ± 0.2	>2.9
Percent reduction (%)	–	90.0	>99.8	>99.8
Intermittent irradiation				
PFU (PFU/ml)[a]	$9.7\ (\pm 2.8) \times 10^4$	$1.2\ (\pm 0.3) \times 10^4$	$<1.0 \times 10^2$	$<1.0 \times 10^2$
Log reduction[b]	–	0.9 ± 0.1	>2.9	>2.9
Percent reduction (%)	–	87.4	>99.8	>99.8

[a]Numbers in the parentheses represent the standard deviation (SD).

[b]Data are presented as the mean ± SD.

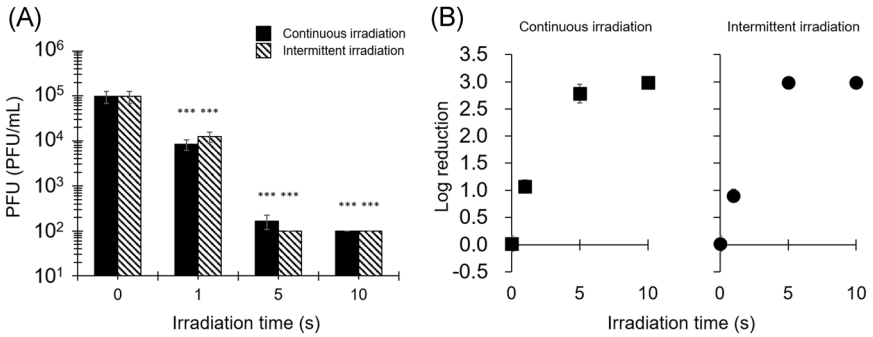

FIGURE 1 Inactivating effect of deep-ultraviolet light-emitting diode (DUV-LED) irradiation on *Dabie bandavirus* severe fever with thrombocytopenia syndrome virus (SFTSV) infectivity. (A) Reduction in the number of SFTSV plaque forming units (PFU) induced by DUV-LED irradiation according to irradiation time. Asterisks indicate a significant difference in PFU (PFU/ml) between the irradiation and control groups (0 s irradiation group) (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). (B) Viral inactivation, expressed as log reduction. Values are presented as the mean ± standard deviation

a potential solution for controlling SFTSV, and possibly other infectious agents.

Although this study demonstrated significant inactivation of SFTSV using DUV-LED, it has some limitations. First, the distance from the irradiation port to the bottom of the Petri dish was limited to 20 mm. This was established assuming that the device would be applied to sterilization of contaminated surface which is adjacent from the irradiation port, such as floor cleaner and hand-held sterilizer. Therefore, the effects reported herein may be limited to these conditions. Other parameters, such as current, frequency, and duty rate, may need to be altered to effectively sterilize objects at a greater distance. Second, this study employed DUV of a single wavelength (280 ± 5 nm) and did not verify the mechanism by which SFTSV was inactivated. This study used an LED device to deliver the DUV radiation due to its long life, easy handling, cost-effectiveness, and smaller environment impact. The wavelength used in this study (280 ± 5 nm) was selected based on practicality because it has high output (radiation) power and increases the durability of the LED device, resulting in higher sterilization power. DUV has a range of wavelengths that are similar to the absorption wavelengths of nucleic acids; thus direct damage to the viral genome is likely to be the primary cause of inactivation after DUV irradiation.[18] Wavelength of 253.7 nm inactivates virus by damaging the viral genome,[13] although the degree of inactivation is influenced by the differences in wavelengths.[19] While 253.7 nm DUV damages the genome but does not degrade viral proteins,[13] wavelengths between 210 and 240 nm emmitted by krypton chloride (KrCl*) excimers or tunable lasers provide greater inactivation, possibly by damaging both viral proteins and nucleic acids.[19,20] Further studies are needed to verify the effects of different radiation wavelengths on SFTSV inactivation; the results of such studies will enable selection of optimal conditions for different situations and environments.

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CONFLICTS OF INTEREST

H. S. receives part of his salary from Nikkiso Co., Ltd., Tokyo, Japan. Nikkiso Co., Ltd. supplied the DUV-LED apparatus used in this study. Nikkiso Co., Ltd. played no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The remaining authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: Akatsuki Saito and Tamaki Okabayashi. *Methodology*: Chiho Kaneko, Akatsuki Saito, Hiroko Inagaki, Hironobu Sugiyama, Shouichi Fujimoto, and Tamaki Okabayashi. *Formal analysis*: Chiho Kaneko. *Investigation*: Chiho Kaneko, Akatsuki Saito, Eugene Mazimpaka, and Tamaki Okabayashi. *Resources*: Hiroko Inagaki, Hironobu Sugiyama, and Tamaki Okabayashi. *Writing—Original draft*: Chiho Kaneko. *Writing—Review and editing*: Chiho Kaneko, Akatsuki Saito, Hiroko Inagaki, Hironobu Sugiyama, Eugene Mazimpaka, Shouichi Fujimoto, and Tamaki Okabayashi. *Funding acquisition*: Chiho Kaneko, Akatsuki Saito, and Tamaki Okabayashi.

DATA AVAILABILITY STATEMENT

All relevant data are shown within the manuscript.

REFERENCES

1. Kuhn JH, Adkins S, Alioto D, et al. 2020 taxonomic update for phylum *Negarnaviricota* (*Riboviria*: *Orthornavirae*), including the large orders *Bunyavirales* and *Mononegavirales*. *Arch Virol*. 2020;165(12):3023-3072. doi:10.1007/s00705-020-04731-2
2. Niu G, Li J, Liang M, et al. Severe fever with thrombocytopenia syndrome virus among domesticated animals, China. *Emerg Infect Dis*. 2013;19(5):756-763. doi:10.3201/eid1905.120245
3. Yu XJ, Liang MF, Zhang SY, et al. Fever with thrombocytopenia associated with a novel Bunyavirus in China. *N Engl J Med*. 2011;364(16):1523-1532. doi:10.1056/NEJMoa1010095
4. Hayasaka D, Fuxun Y, Yoshikawa A, et al. Seroepidemiological evidence of severe fever with thrombocytopenia syndrome virus infections in wild boars in Nagasaki, Japan. *Trop Med Health*. 2016;44(1):6. doi:10.1186/s41182-016-0009-6
5. Kim KH, Yi J, Kim G, et al. Severe fever with thrombocytopenia syndrome, South Korea, 2012. *Emerg Infect Dis*. 2013;19(11):1892-1894. doi:10.3201/eid1911.130792
6. Lin TL, Ou SC, Maeda K, et al. The first discovery of severe fever with thrombocytopenia syndrome virus in Taiwan. *Emerg Microbes Infect*. 2020;9(1):148-151. doi:10.1080/22221751.2019.1710436
7. Takahashi T, Maeda K, Suzuki T, et al. The first identification and retrospective study of severe fever with thrombocytopenia syndrome in Japan. *J Infect Dis*. 2014;209(6):816-827. doi:10.1093/infdis/jit603
8. Tran XC, Yun Y, An LV, et al. Endemic severe fever with thrombocytopenia syndrome, Vietnam. *Emerg Infect Dis*. 2019;25(5):1029-1031. doi:10.3201/eid2505.181463
9. Center for Field Epidemic Intelligence, Research and Professional Development, Infectious Disease Surveillance Center, & Department of Virology 1. Severe fever with thrombocytopenia syndrome (SFTS). National Institute of Infectious Diseases. July 2021. Accessed October 24, 2021. https://www.niid.go.jp/niid/ja/sfts/3143-sfts.html
10. Yamanaka A, Kirino Y, Fujimoto S, et al. Direct transmission of severe fever with thrombocytopenia syndrome virus from domestic cat to veterinary personnel. *Emerg Infect Dis*. 2020;26(12):2994-2998. doi:10.3201/eid2612.191513
11. Gai Z, Liang M, Zhang Y, et al. Person-to-person transmission of severe fever with thrombocytopenia syndrome Bunyavirus through blood contact. *Clin Infect Dis*. 2012;54(2):249-252. doi:10.1093/cid/cir776
12. Kirino Y, Ishijima K, Miura M, et al. Seroprevalence of severe fever with thrombocytopenia syndrome virus in small-animal veterinarians and nurses in the Japanese prefecture with the highest case load. *Viruses*. 2021;13(2):229. doi:10.3390/v13020229
13. Lo CW, Matsuura R, Iimura K, et al. UVC disinfects SARS-CoV-2 by induction of viral genome damage without apparent effects on viral morphology and proteins. *Sci Rep*. 2021;11(1):13804. doi:10.1038/s41598-021-93231-7
14. Inagaki H, Saito A, Kaneko C, Sugiyama H, Okabayashi T, Fujimoto S. Rapid inactivation of SARS-CoV-2 variants by continuous and intermittent irradiation with a deep-ultraviolet light-emitting diode (DUV-LED) device. *Pathogens*. 2021;10(6):754. doi:10.3390/pathogens10060754
15. Song K, Taghipour F, Mohseni M. Microorganisms inactivation by continuous and pulsed irradiation of ultraviolet light-emitting diodes (UV-LEDs). *Chem Eng J*. 2018;343:362-370. doi:10.1016/j.cej.2018.03.020
16. Sato Y, Mekata H, Sudaryatma PE, et al. Isolation of severe fever with thrombocytopenia syndrome virus from various tick species in area with human severe fever with thrombocytopenia syndrome cases. *Vector Borne Zoonotic Dis*. 2021;21(5):378-384. doi:10.1089/vbz.2020.2720
17. R Core Team R: a language and environment for statistical computing. R Foundation for Statistical Computing; 2021.
18. Beck SE, Rodriguez RA, Hawkins MA, Hargy TM, Larason TC, Linden KG. Comparison of UV-induced inactivation and RNA damage in MS2 phage across the germicidal UV spectrum. *Appl Environ Microbiol*. 2016;82(5):1468-1474. doi:10.1128/AEM.02773-15
19. Ma B, Gundy PM, Gerba CP, Sobsey MD, Linden KG, Dudley EG. UV inactivation of SARS-CoV-2 across the UVC spectrum: KrCl* excimer, mercury-vapor, and light-emitting-diode (LED) sources. *Appl Environ Microbiol*. 2021;87(22):e01532-01521. doi:10.1128/AEM.01532-21
20. Beck SE, Rodriguez RA, Linden KG, Hargy TM, Larason TC, Wright HB. wavelength dependent UV inactivation and DNA damage of adenovirus as measured by cell culture infectivity and long range quantitative PCR. *Environ Sci Technol*. 2014;48(1):591-598. doi:10.1021/es403850b

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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