LETTER

Rapid inactivation of SARS-CoV-2 with Deep-UV LED irradiation

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Abstract

The spread of novel coronavirus disease 2019 (COVID-19) infections worldwide has raised concerns about the prevention and control of SARS-CoV-2. Devices that rapidly inactivate viruses can reduce the chance of infection through aerosols and contact transmission. This in vitro study demonstrated that irradiation with a deep ultraviolet light-emitting diode (DUV-LED) of 280 ±5 nm wavelength rapidly inactivates SARS-CoV-2 obtained from a COVID-19 patient. Development of devices equipped with DUV-LED is expected to prevent virus invasion through the air and after touching contaminated objects.

Letter

The novel coronavirus SARS-CoV-2 pandemic has spread worldwide and placed countries in emerging, rapidly transforming situations. The World Health Organization (WHO) clarified that more than 5.3 million cases of COVID-19 and 342,000 deaths had been reported to WHO by 25 May 2020 [1]. Infectious virus is detected in specimens from the respiratory tract, nasopharyngeal sites, and feces in COVID-19 patients [2]. Recently, infectious SARS-CoV-2 was isolated from the urine of a COVID-19 patient [3]. SARS-CoV-2 is detectable in aerosols for up to 3 h, up to 4 h on copper, up to 24 h on cardboard and up to 2–3 days on plastic and stainless steel [4]. To prevent exposure to contaminated material (contact infection), which is one of the major transmission routes, hand hygiene with alcohol is recommended, but its effectiveness in preventing the spread of SARS-CoV-2 infection may be insufficient [5, 6].

A deep ultraviolet light-emitting diode (DUV-LED) instrument generating around 250–300 nm wavelength has been reported to effectively inactivate microorganisms, including bacteria, viruses and fungi [7–10], but effects on SARS-CoV-2 have not been reported. We evaluated the antiviral efficacy of irradiation by DUV-LED, generating the narrow-range wavelength (280±5 nm) (Nikkiso Co., Tokyo, Japan), against SARS-CoV-2.

A strain of SARS-CoV-2 isolated from a patient who developed COVID-19 in the cruise ship Diamond Princess in Japan in February 2020 [11] was obtained from the Kanagawa Prefectural Institute of Public Health (SARS-CoV-2/Hu/Dp/Kng/19-027, LC528233). The virus was propagated in Vero cells cultured in minimum essential medium (MEM) containing 2% fetal bovine serum (FBS). At 48 h after infection, virus stocks were collected by centrifuging the culture supernatants of infected Vero cells at 3,000 rpm for 10 min. Clarified supernatants were kept at -80 °C until use. Aliquots of stock virus were diluted with phosphate-buffered saline and adjusted to 2.0 ×10⁴ plaque-forming units (PFU)/ml. For the evaluation of DUV-LED inactivation, aliquots of virus stock (150 µl) were placed in the center of a 60-mm Petri dish and irradiated with 3.75 mW/cm² at work distance 20 mm for a range of times (n=3 each for 1, 10, 20, 30, or 60 s). Each virus stock irradiated with DUV-LED was serially diluted in 10-fold steps, then inoculated onto Vero monolayers in a
12-well plate. After adsorption of virus for 2 h, cells were overlaid with MEM containing 1% carboxymethyl cellulose and 2% FBS (final concentration). Cells were incubated for 72 h in a CO₂ incubator, then cytopathic effects were observed under a microscope. An unirradiated virus suspension was used as a negative control. To calculate PFU, cells were fixed with 10% formalin for 30 min, followed by staining with 0.1% methylene blue solution. The antiviral effects of DUV-LED irradiations were assessed using the logPFU ratio, calculated as logPFU ratio=\log_{10}(N_t/N_0), where N_t is the PFU count of the UV-irradiated sample, and N_0 is the PFU count of the sample without UV irradiation. In addition, the infectious titer reduction rate was calculated as \((1-1/10^{\log \text{PFU ratio}}) \times 100\) (%). All experiments were performed in a BSL-3 laboratory.

We observed a marked cytopathic effect in virus-infected cells without DUV-LED irradiation (Figure 1A, see “0 s”). In contrast, virus-infected cells irradiated for 60 s showed largely comparable morphology to mock cells (Figure 1A, see “60 s”). To our surprise, virus-infected cells irradiated for 1 s showed minimal change (Figure 1A, see “1 s”). The plaque assay (Figure 1B) revealed that short time DUV-LED irradiation rapidly inactivated SARS-CoV-2 (Figure 1C and Table S1). Of note, the infectious titer reduction rate of 87.4% was already recognized with irradiation of virus stock for 1 s, and the rate was 99.9% with irradiation for 10 s. These results suggest that DUV-LED drastically inactivated SARS-CoV-2 with irradiation for even a very short time.

UV-LEDs providing irradiation at various peak emission wavelengths, such as UV-A (320–400 nm), UV-B (280–320 nm), and UV-C (100–280 nm), have been adopted to inactivate various pathogenic species, including bacteria, viruses and fungi. Devices equipped with UV-LEDs are now beginning to be introduced into medical fields. UV-C is considered to be the most effective germicidal region of the UV spectrum, acting through the formation of photoproducts in DNA [12]. These pyrimidine dimers interrupt transcription, translation and replication of DNA, eventually leading to inactivation of microorganisms [13]. The efficacy of this inactivation may depend not only on the wavelength, but also on factors such as the target (e.g., bacterial species), light output and environmental conditions. The DUV-LED we used has the characteristics of a narrow-range wavelength and high power for short exposure times and long-term use. This study demonstrated for the first time the rapid inactivation of SARS-CoV-2 under DUV-LED irradiation. As shown in Figure 1B, cytopathic effects were observed in control Vero cells infected with SARS-Cov-2, but not in these cells with DUV-LED irradiation for only 10 s. As well as in community settings, healthcare settings are also vulnerable to the invasion and spread of SARS-CoV-2, and the stability of SARS-CoV-2 in aerosols and on surfaces [4] likely contributes to virus transmission in medical environments. No vaccines, neutralizing antibodies, or drugs are currently available for prevention and treatment of SARS-CoV-2. By revealing that SARS-Cov-2 inactivation can be achieved with very short-term DUV-LED irradiation, this study provides useful baseline data toward securing a safer medical environment. Development of devices equipped with DUV-LED is expected to prevent
the virus invasion through the air and after touching contaminated objects.

**Contributors**

H.I. and H.S. conceived the study and wrote the manuscript. A.S. and T.O. conducted the experiments dealing with viruses. S.F. contributed to the study design, study supervision and manuscript revision.

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**Declaration of interest statement**

H.S. receives part of his salary from Nikkiso Co., Ltd., Tokyo, Japan. Nikkiso supplied the deep ultraviolet light-emitting diode (DUV-LED) instrument for evaluation. Nikkiso had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The other authors declare no conflicts of interest.

**Figure 1.** Inhibitory effects of DUV-irradiation on SARS-CoV-2.

(A) Cytopathic changes in virus-infected Vero cells without DUV-LED irradiation (0 s), or with DUV-LED irradiation for 1, 10, 20, 30 or 60 s.

(B) Plaque formation in Vero cells. Virus solutions irradiated with DUV-LED for several durations were diluted (100-fold) and inoculated to Vero cells. A representative result is shown.

(C) Time-dependent inactivation of SARS-CoV-2 by DUV-LED irradiation. The results shown are the mean and standard deviation (SD) of triplicate measurements. The dashed line indicates the limit of detection.

**Table S1.** Differences in infectious titer with different DUV-LED irradiation times.

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Figure 1

B

Mock

0 s

1 s

60 s

C

![Log PFU/mL vs. DUV-LED irradiation time (s)](image)

- **DUV-LED irradiation time (s)**
  - 0 s
  - 1 s
  - 60 s

- **Log PFU/mL**
  - 0
  - 1
  - 2
  - 3
  - 4
  - 5
Supplement Table 1

Differences in infectious titer with different DUV-LED irradiation times.

| Irradiation time | control (no irradiation) | DUV-LED irradiation time |
|------------------|--------------------------|--------------------------|
|                  |                          | 1 sec | 10 sec | 20 sec | 30 sec | 60 sec |
| PFU(PFU/mL)      | 3.7x10^4                 | 4.7x10^3 | 2.7x10^1 | 6.7x10^0 | <20 | <20 |
| Log PFU ratio 1) | —                        | 0.9 | 3.1 | >3.3 | >3.3 | >3.3 |
| Infection titer reduction ratio 2)(%) | — | 87.4 | 99.9 | >99.9 | >99.9 | >99.9 |

1) \( \log_{10} (Nt/N0) \), where \( Nt \) is the PFU count of the UV-irradiated sample, and \( N0 \) is the PFU count of the sample without UV irradiation. 2) \( (1-1/10^{\log \text{PFU ratio}}) \times 100 \) (%).