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Short Communication

Clinical application of 222 nm wavelength ultraviolet C irradiation on SARS CoV-2 contaminated environments

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Abstract This was a preliminary study on ultraviolet C (UVC) irradiation for SARS-CoV-2-contaminated hospital environments. Forty-eight locations were tested for SARS-CoV-2 using RT-PCR (33.3% contamination rate). After series dosages of 222-nm UVC irradiation, samples from the surfaces were negative at 15 s irradiation at 2 cm length (fluence: 81 mJ/cm^2).

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Introduction

Coronavirus infection disease-19 (COVID-19) continues to be a pandemic in 2021. Environmental sodium hypochlorite cleaning and patients wearing masks effectively prevent nosocomial infections in healthcare workers (HCWs).[1] However, sodium hypochlorite cleaning still needs HCWs to enter the contaminated room, which posesa risk of nosocomial infection and could be resolved by automated disinfection such as harmless ultraviolet C (UVC). The krypton-chlorine (Kr—Cl) excimer lamp produces 222-nm UVC irradiation, and the highest exposure is 500 mJ/cm^2, which is safe in human skin models.[2] This study aimed to use a Kr—Cl excimer lamp to reproduce the viral inactive effect on SARS-CoV-2 in hospital-contaminated environments.

Methods

This COVID-19 environmental study was approved by the Institutional Review Board of the Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, on July 28, 2021 (Protocol No.: 10-X-101).

Three patients with SARS CoV-2 infection with a nasal swab reverse transcriptase-polymerase chain reaction (RT-PCR) cycle threshold values (CtV) lower than 25 were selected for the environmental contamination study. Patient 1 was a 64-year-old man, patient 2 was a 45-year-old man, and patient 3 was a 37-year-old man. All presented with simple SARS-CoV-2 bronchopneumonia with a nasal swab SARS-CoV-2 RT-PCR CtV of 18, 16, and 23, respectively. Each patient was informed that they did not need to wear a surgical mask in their isolation room during the study. The environmental study time was on the day after admission and 48—72 h. The patient performed the usual daily activity without any environmental cleaning. The most frequently touched surfaces in their single independent ward of the hospital were selected with 12 points.

Environmental samples were collected before 222-nm UVC irradiation, and after 5, 10, 15, and 20s, for SARS-CoV2 RT-PCR testing to establish routine disinfection procedures. Ward temperature was 24.1—30.8 °C, and relative humidity was 56.5—68.3%.

All environmental samples were collected using sterile nasal swabs (LIBO Specimen Collection and Transport Swabs, LIBO Medical Products Inc., Taiwan) and were transported in a viral transport medium (CMP®, Taiwan). A sample from each equipment surface was collected at three different positions to increase the positive sampling rate.

All samples were analyzed for SARS-CoV-2 using RT-PCR detection at a biosafety level 2 (BSL-2) laboratory with a built-in negative-pressure room for immediate RT-PCR testing. Specific RT-PCR targeting of RNA-dependent RNA polymerase (*RdRP*), *E*, and *N* genes was used to detect the presence of SARS-CoV-2.[3] The samples were stored at 4 °C and tested within 2 days if the environmental samplings were routine sampling. The procedure of nucleic acid extraction was described in supplementary documents. For the *E* and *N* genes, a 25 μL-reaction was performed with 6 μL of template RNA and 19 μL of the PCR reaction master mix, and samples were detected using a LabTurbo™ AIO COVID-19 RNA testing kit (Cat. No. Acov11240) on a LabTurbo™ 48 AIO (Lab Turbo Biotech Co., Ltd., Taiwan). Thermal cycling was performed at 55 °C for 10 min for reverse transcription, followed by heat activation at 95 °C for 1 min, followed by 45 cycles of amplification at 95 °C for 10 s (denaturation) and 60 °C for 15 s (annealing/extension). Positive findings were defined as the least significant Ct of *E* or *N* genes. The human RNAse P (RNP) gene was used for nucleic acid extraction procedural control and secondary negative control. The number of cycles required for the fluorescent signal to cross the threshold in RT-PCR was analyzed. In general, lower Ct values are negative, corresponding to higher viral loads.

We used 222-nm UVC irradiation for environmental cleaning simulation. The Care222® U+ handheld lamp (USHIO Inc., Taiwan and Delta Electronics, Inc., Taiwan) emits 222-nm UVC at 27mJ/cm2 after 5 s irradiation in a single room with a fixed length of 2 cm with stabilized UV radiation intensity. For the purpose of the dosage-related cleaning effect, the irradiation times were 5, 10, 15, 20 s. The fluences were 27, 54, 81, 108 mJ/cm^2. Environmental samples were collected again after radiation.

Statistical analysis

Continuous data are expressed as the mean ± standard error. Categorical data are expressed as frequencies and percentages. The chi-square test was used to compare categorical variables between the different UVC irradiation dosages. The linear trend was also tested using the chi-square test for UVC irradiation of the dose effect on SARS-CoV-2. The data were analyzed using SPSS (version 24.0; IBM Corp., Armonk, NY, USA), and a p-value of <0.05 was considered statistically significant.

Results

Forty-eight environmental samples were collected. The total contamination rate was 33.3% (Fig. S1 and Supplementary document).

To estimate the proper irradiation dosage of 222-nm UVC, a series of studies were conducted to test its dose-related effect on SARS-CoV-2 contaminated environments. In the first survey, four locations in each of the three isolation wards were selected for environmental sampling before irradiation (Table 1). Before irradiation, three (25.0%) were positively contaminated. SARS-CoV-2 was completely inactivated after 15 s of 222-nm UVC irradiation. However, the contamination was still present in three (25.0%) after 5 s of irradiation, and one (8.3%) after 10 s of irradiation, although the decrease was not statistically significant. The separate *E* and *N* genes also decreased after irradiation, although this was not statistically significant. The internal control also decreased slowly from 58.3% before the irradiation to 41.7%, 41.7%, and 25.0% after 5, 10, and 15 s, respectively ($p = 0.433$).

To determine the optimal UVC irradiation dose (fluence) for SARS-CoV-2 environmental disinfection, repeated studies on the dose effect of UVC irradiation were conducted for 5—20 s on the second-day survey of regular

Table 1 The dosage effect of 222-nm UVC radiation at various irradiation periods.

Irradiation time	Control Before irradiation	Irradiation for 5 s	Irradiation for 10 s	Irradiation for 15 s	Irradiation for 20 s	p-value	p-value[a]
1st survey (n = 12)							
Positive rate	3 (25.0%)	3 (25.0%)	1 (8.3%)	0 (0%)		0.211	0.127
E gene, n (%)	2 (16.7%)	1 (8.3%)	0 (0%)	0 (0%)		0.271	0.065
CtV, mean ± SE	32.6 ± 0.5	35.1	NA	NA		0.221[b]	
RNA, copies/mL	2716.5 ± 716.5	500	NA	NA		0.325	
N gene, n (%)	3 (25.0%)	3 (25.0%)	1 (8.3%)	0 (0%)		0.211	0.127
CtV, mean ± SE	32.7 ± 1.2	32.9 ± 0.7	32.3	NA		0.867[b]	
RNA, copies/mL	1208.7 ± 836.4	732.7 ± 387.4	858	NA		0.875	
Internal control, n (%)	7 (58.3%)	5 (41.7%)	5 (41.7%)	3 (25.0%)		0.433	0.136
CtV, mean ± SE CtV (ean±SE)	32.5 ± 0.9	32.5 ± 1.0	33.2 ± 0.7	34.5 ± 0.7		0.453[b]	
2nd survey (n = 36)							
Positive rate	13 (36.1%)	3 (8.3%)	2 (5.6%)	0 (0%)	0 (0%)	<0.001*	<0.001*
E gene, n (%)	6 (16.7%)	2 (5.6%)	2 (5.6%)	0 (0%)	0 (0%)	0.013*	<0.001*
CtV, mean ± SE	28.0 ± 2.3	32.9 ± 1.8	33.5 ± 0.5	NA	NA	0.316[b]	
RNA, copies/mL	$9.5 \times 10^6 \pm 9.3 \times 10^6$	$4.6 \times 10^3 \pm 4.0 \times 10^3$	$3.5 \times 10^3 \pm 1.4 \times 10^3$	NA	NA	0.756[b]	
N gene, n (%)	13 (36.1%)	2 (5.6%)	2 (5.6%)	0 (0%)	0 (0%)	<0.001*	<0.001*
CtV, mean ± SE	31.0 ± 0.5	32.3 ± 1.6	35.2 ± 0.9	NA	NA	0.066[b]	
RNA, copies/mL	3590 ± 736	1417 ± 1086	156 ± 85	NA	NA	0.163[b]	
Internal control, n (%)	13 (36.1%)	9 (25.0%)	5 (13.9%)	5 (13.9%)	4 (11.1%)	0.043*	0.003*
CtV, mean ± SE CtV (ean ± SE)	30.9 ± 0.8	30.8 ± 3.7	33.4 ± 1.5	34.7 ± 0.6	33.1 ± 1.4	0.071[b]	

[a] For the linear trend testing of 222-nm UVC irradiation dose effect.
[b] For at least three groups, Kruskal–Wallis test.
CtV, cycle threshold value; SE, standard error; NA, not applicable. The categorical variables are presented as n (%), n: presented as the number of positive results of environmental sampling, (%): presented as the percentage of positive rate. The continuous variables are presented as mean ± SE. *, $p < 0.05$.

patient activity (Table 1). SARS-CoV-2 was completely inactivated after 15 s of 222-nm UVC irradiation. The positive contamination rates were 13/36 (36.1%) before irradiation and 3/36 (8.3%), 2/36 (5.6%), 0%, and 0% after 5, 10, 15, and 20 s irradiation, respectively ($p < 0.001$). The separate *E* and *N* genes showed the same decreasing trend as the contamination rate from 16.7% to 36.1% before irradiation to 0% after 15 s irradiation. The 15 s in 2 cm length had a fluence of 81 mJ/cm^2, which was the optimal 222-nm UVC dose. The internal control was the same 13/36 (36.1%) as the contamination rate of SARS-CoV-2 and slowly decreased with irradiation to 25.0%, 13.9%, 13.9%, and 11.1% after 5, 10, 15, and 20 s of irradiation, respectively ($p = 0.043$).

Discussion

Our preliminary study simulated the 222-nm UVC irradiation effect on the SARS-CoV-2 contaminated surfaces of the hospital isolation ward. The effective fluence was 81 mJ/cm^2 with an environmental SARS-CoV-2 eradication rate of 100%.

In traditional disinfection procedures, patients must leave the ward before 254-nm UVC irradiation to prevent the risk of skin cancer and corneal damage. A SARS CoV2 *in vitro* study showed 1–3 mJ/cm^2 222-nm UV irradiation reduced 88.5%–99.7% of the SARS CoV2 virus detected as 50% tissue culture infectious dose (TCID 50).[3] In animal studies, 222-nm UV light did not damage the hairless mouse skin.[4] Another hairless mouse skin study found no damage to the skin after 10 days of 450 mJ/cm^2/day chronic irradiation.[5] The corneas of Sprague Dawley albino rats were not damaged by 222-nm UV light with a high exposure level of 600 mJ/cm^2. In an *in vitro* study of mammalian skin, 222-nm UV light did not yield UV-associated premutagenic DNA lesions (cyclobutane pyrimidine dimers and 6–4 photoproducts).[4] The other simulated mice study showed Xpa knockout mice irradiated with a high dose of 222-nm UVC, and there was no skin tumor formation.[6] In a clinical trial of 20 healthy humans, 222-nm UV (500 mJ/cm^2) was found to be safe and have a bactericidal effect on the human skin after 3 months follow-up.[7] Therefore, 222-nm UVC has potential safety in a clean environment and prevents skin damage compared to 254-nm UVC; however, further studies are needed.

Far-UVC light (222 nm) with low doses of 1.7 and 1.2 mJ/cm^2 could inactivate 99.9% of aerosolized coronavirus 229E and OC 43, respectively.[8] Light at 222 nm also inactivates >95% aerosolized H1N1 influenza virus with a low dose of 2 mJ/cm^2 [9]. Another study simulated irradiation for different UV fluences; continuous and intermittent irradiation had no differing effects on SARS CoV2.[10]

Thus, 222-nm UV light irradiation is safe; it neither induces skin cancer nor corneal damage in animal or human skin studies. The bactericidal effect and killing of

coronaviruses have been tested successfully in safety fluences. The maximum safety fluence was 500 mJ/cm^2 [2,7]. The limitation of the study was that the contaminated location could not be detected immediately before the SARS-CoV-2 RT-PCR results are reported.

In conclusion, 222-nm UVC light irradiation in a hospital ward environment contaminated with SARS CoV-2 is effective, with a fluence of 81 mJ/cm^2 (2 cm in length, 15 s). Further design of 222-nm UVC equipment would aid in regular environmental disinfection procedures during the pandemic.

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Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2021.12.005.