

Article

The Effectiveness of Far-Ultraviolet (UVC) Light Prototype Devices with Different Wavelengths on Disinfecting SARS-CoV-2

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Abstract: Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has emerged as a serious threat to human health worldwide. The inactivation of SARS-CoV-2 on object surfaces and in the indoor air might help to halt the COVID-19 pandemic. Far-ultraviolet light (UVC) disinfection has been proven to be highly effective against viruses and bacteria. To understand the wavelength and duration of UVC radiation required for SARS-CoV-2 inactivation, we examined the efficacy of UVC light prototype devices with the wavelengths of 275, 254, and 222 nm. The disinfection effectiveness was determined by cell-based assays including the median tissue culture infectious dose (TCID₅₀) and an immunofluorescent assay on African green monkey kidney epithelial Vero E6 cells. Among the three prototypes, the UVC LED (275 nm) had the best virucidal activity with a log-reduction value (LRV) >6 after 10 s of exposure. The mercury lamp (254 nm) reached similar virucidal activity after 20 s of exposure. However, the excimer lamp (222 nm) showed limited anti-SARS-CoV-2 activity with a LRV <2 after 40 s of exposure. Overall, in comparison, the UVC LED (275 nm) exhibited superior SARS-CoV-2 disinfection activity than the mercury lamp (254 nm) and the excimer lamp (222 nm).

Keywords: SARS-CoV-2; UVC light; disinfection; environmental contamination

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1. Introduction

COVID-19, a disease caused by SARS-CoV-2, has caused a serious pandemic that necessitated significant scientific, economic, and public efforts to control the threat [1–4]. The most effective way to halt the pandemic is to prevent the spread by disrupting the viral transmission routes. Pathogens, such as viruses, can transmit and spread through the atmosphere or contact with a contaminated surface. Many methodological approaches, such as heat sterilization, chemical disinfectant, ventilation, and ultraviolet (UV) irradiation can help to reduce the risk of viral infection [5]. Over the last few decades, UV-based disinfection has become a common chemical-free technology [6]. UV radiation is extremely effective at disinfecting bacteria in various mediums, such as water, air, and an object's surface [7]. UVC light air and surface disinfection have attracted considerable attention, and several products have been released in the market since the COVID-19 pandemic began. UV surface disinfection systems have been implemented in a wide range of public places, including hospitals and health care institutions, as well as restaurants and cafeterias [8]. However, a lack of understanding of the critical features of UV disinfection

has resulted in an inappropriate use of this formidable platform, not only by the general public, but also by some UV surface disinfection manufacturers.

UV light occurs in a multitude of forms, each of which is classified according to the amount of energy it possesses. UVA light is the one with the least amount of energy and is less effective at inactivating SARS viruses than UVB or UVC radiation [9]. UVB light, located in the center of the UV spectrum, is known to damage DNA and is related to the development of skin cancer and cataracts. UVC light is the most powerful and effective form of UV light for disinfecting surfaces, air, and liquids since it has the highest energy. The UVC light can be effectively absorbed by the biomolecules, i.e., nucleic acid basis or proteins, leading to the generation of photoproducts that inactivate the viruses, hence it is very effective in disinfecting surfaces, air, and liquids. UVC light causes nucleic acids and proteins to break down, which kills germs including viruses and bacteria [5]. The reason why UV radiation is effective in disinfection is because it has enough energy to break DNA chemical bonds. UVC light, which has a wavelength range of 260 nm to 275 nm, damages the genetic information stored in DNA, rendering dangerous microbes like bacteria and viruses ineffective. Pathogens such as viruses and bacteria also require DNA and RNA to survive, and without this genetic material, these pathogens are unable to replicate, leading to the death of an infectious colony. Since the intercellular components of bacteria, such as RNA, DNA, and proteins can sensitively absorb UVC photons, the UVC range has a greater adverse effect on microbial cells. UVC photons that are absorbed cause significant damage to microorganisms' genomic systems, such as nucleic acid and microorganismal proteins, preventing them from replicating and surviving. This is due to the collapse of the adenine–thymine bond, which results in the formation of a covalent linkage, the pyrimidine dimer, between two adenines, preventing the cell from replicating. UVC light has been used to destroy a significant amount of SARS-CoV-2 in liquid culture after 9 min of exposure [10]. Another study used far-UV light radiation to disinfect SARS-CoV-2 surface contamination and found that 222 nm UVC light reduced the viable SARS-CoV-2 by 99.7% in 30 s [11]. Far-UVC light also killed 99.9% of airborne human common cold coronaviruses, 229E and OC43, in around 25 min [12].

A variety of UVC sources have been employed, including low and medium pressure mercury UV lamps, UV light-emitting diodes (UV-LEDs), and far-UVC (200–240 nm) radiating excimer and micro plasma lamps [13–15]. Pulsed xenon lamps also emit a brief pulse of broad spectrum (UV, visible, and infrared) light that has been filtered to release predominantly UVC radiation, and are used to treat environmental surfaces in operating rooms and other spaces in hospitals [16]. Although there have been a few reports on the efficiency of UV disinfection on SARS-CoV-2, the effect of different wavelengths of UVC on SARS-CoV-2 is unclear. In this study, we compared the effectiveness of UVC light prototype devices with wavelengths of 275, 254, and 222 nm in disinfecting SARS-CoV-2.

2. Materials and Methods

Cells, virus and chemicals. Vero E6 cells (African green monkey kidney epithelial cells, ATCC CRL-1586) purchased from ATCC were propagated in Dulbecco's modified Eagle's minimum essential medium (DMEM, Corning) supplemented with 10% fetal bovine serum (FBS). SARS-CoV-2 (TCDC#4, hCoV-19/Taiwan/4/2020) was provided by the Taiwan CDC and amplified by infecting Vero E6 cells in a biosafety level 3 facility. The culture supernatant was harvested when the cytopathic effects were fully developed. The virus titer was determined by the standard 50% tissue culture infectious dose (TCID₅₀) method and expressed as TCID₅₀/mL. The human anti-SARS-CoV-2 N protein antibody was kindly provided by Dr. An-Suei Yang (Genomics Research Center, Academia Sinica).

Disinfection test. Three UV light prototype devices, a UVC LED (275 nm), a mercury lamp (254 nm), and an excimer lamp (222 nm), designed and produced by Everlight Electronics, were used in this disinfection test. The UV devices were positioned 11 cm above the SARS-CoV-2-containing medium and the radiation intensities of the UVC LED (275 nm), the mercury lamp (254 nm), and the excimer lamp (222 nm) at the medium's surface

were measured by UVR-300 (Topcon, Japan) as 79.8, 850, and 7.0 $\mu\text{W}/\text{cm}^2$, respectively. The depth of the medium was 1 cm, and the radiation intensities of the UVC LED (275 nm), mercury lamp (254 nm), and excimer lamp (222 nm) at the medium's bottom were 28.9, 305.1, and 1.6 $\mu\text{W}/\text{cm}^2$, respectively. Table 2 shows the UV light accumulative exposure of the three prototype devices. After irradiation, the viral samples were 10-fold serially diluted and added to Vero E6 cells for 4-day incubation. The cells were then fixed with 10% formaldehyde overnight and stained with 0.5% crystal violet for 20 min. After washing, the plates were scored for infection. The Reed and Muench Method [17] was used to determine the virus titer as 50% tissue culture infectious dose per ml ($\text{TCID}_{50}/\text{mL}$).

Immunofluorescent assay. To verify viral infectivity, we also used an immunofluorescent assay to detect SARS-CoV-2 N protein expression. Briefly, the irradiated samples were added to Vero E6 cells for 1 day infection. Cells were fixed and immunostained with an anti-SARS-CoV-2 N protein antibody plus a goat anti-human IgG-Alexa Fluor 488 (A11013, Invitrogen). In addition, the cell nucleus was stained with DAPI (4', 6-Diamidino-2-phenylindole dihydrochloride, D1306, Invitrogen). The signal was observed and photographed by using an immunofluorescent microscope.

Calculation of disinfection ability. The Log reduction value (LRV) for the virus titer was calculated using the formula: $\text{LRV} = \text{Log}_{10}(\text{input virus}) - \text{Log}_{10}(\text{output virus})$. The term "Log reduction" is a mathematical expression for the relative number of living microbes that are eliminated by disinfection. A 1-Log reduction means 90% of a target microbe is inactivated with a 10-fold reduction in the microbe count. A 2-Log reduction results in a 99% reduction, or a 100-fold reduction in microbe count, and so on.

3. Results

For the UVC LED (275 nm) disinfection test, the untreated SARS-CoV-2 titer ($10^7 \text{ TCID}_{50}/\text{mL}$) was reduced to $10^{2.77} \text{ TCID}_{50}/\text{mL}$ after 5 s of irradiation. The viral titer was further reduced to below the detection limit ($10^1 \text{ TCID}_{50}/\text{mL}$) after longer UVC LED exposure for 10, 20, and 40 s (Figure 1). The Log reduction values (LRVs) were 4.23, >6, >6, and >6 for 5, 10, 20, and 40 s of irradiation, respectively (Table 1).

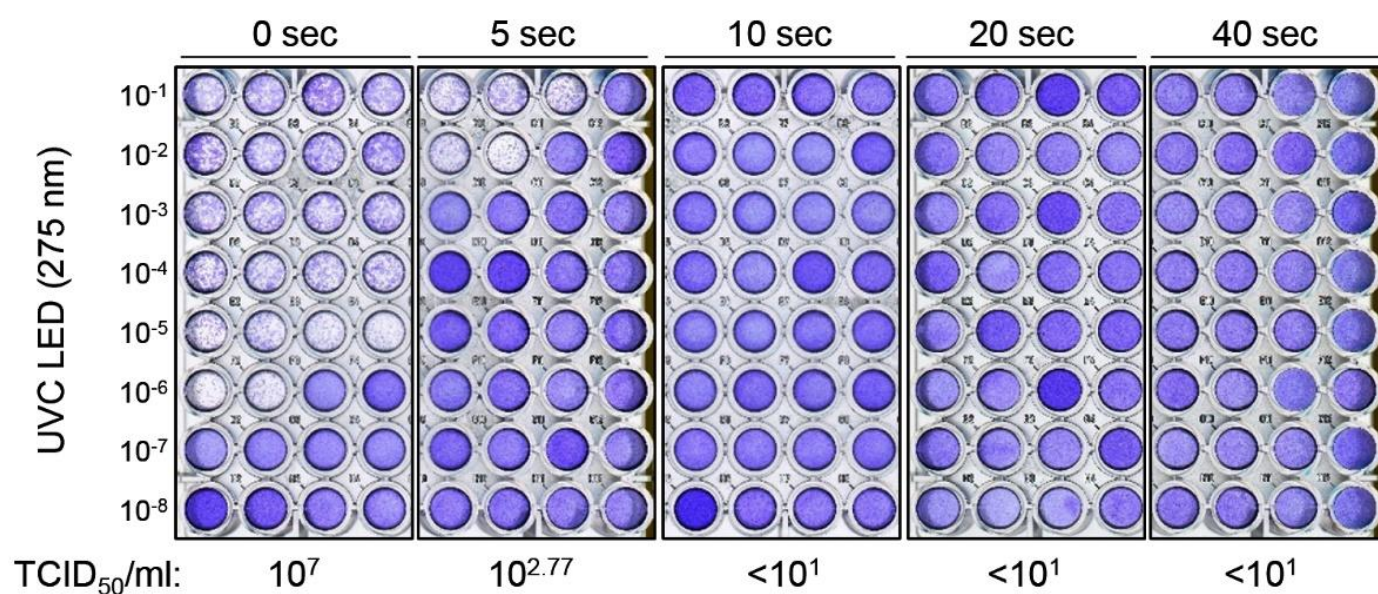


Figure 1. Disinfection efficacy of UVC LED (275 nm) against SARS-CoV-2 was determined by tissue culture infection dose. SARS-CoV-2-containing medium was irradiated with UVC LED (275 nm) at room temperature for the indicated time periods. The virus-containing medium was 10-fold serially diluted and added to Vero E6 cells for 4-day incubation. Cells were then fixed, stained, and scored for infection. TCID_{50} was calculated by Reed and Muench Method (1938).

For the mercury lamp (254 nm) disinfection test, the untreated SARS-CoV-2 titer ($10^{6.67}$ TCID₅₀/mL) was reduced to $10^{5.5}$ and $10^{3.33}$ TCID₅₀/mL after exposure to a mercury lamp for 5 and 10 s, respectively. After longer treatment for 20 and 40 s, the SARS-CoV-2 titer was further reduced to less than 10^1 TCID₅₀/mL (Figure 2). The LRVs were 1.17, 3.34, >6, and >6 for 5, 10, 20, and 40 s of irradiation, respectively (Table 1).

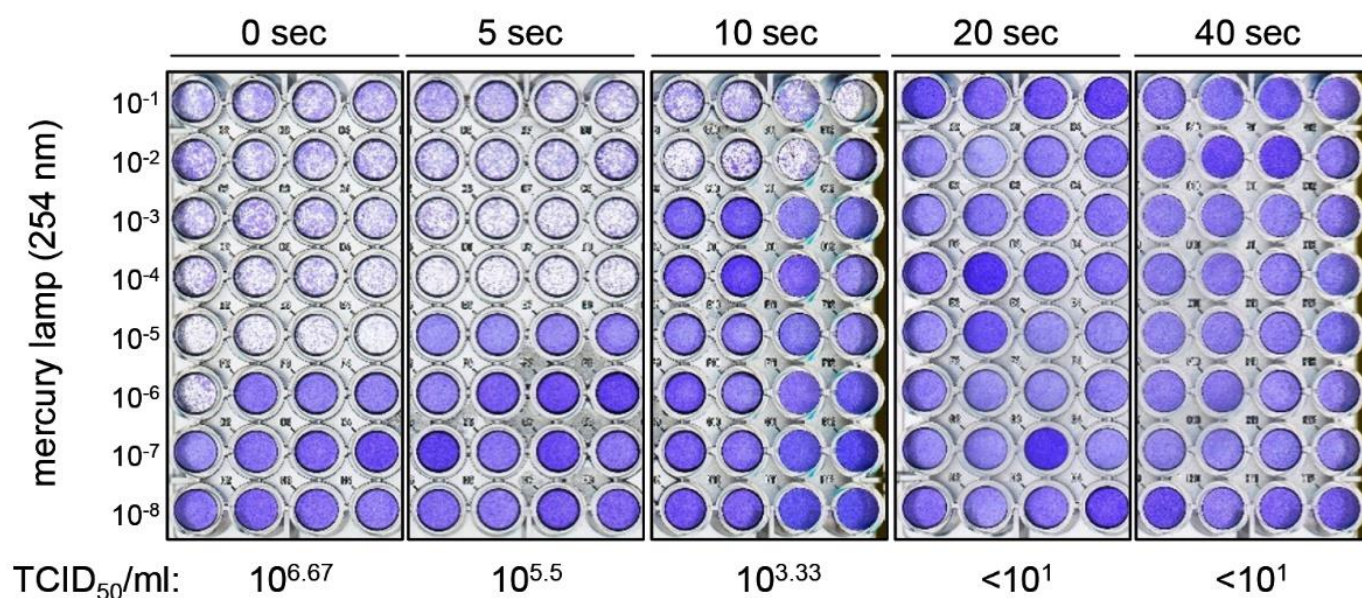


Figure 2. Disinfection efficacy of mercury lamp (254 nm) against SARS-CoV-2 was determined by tissue culture infection dose. SARS-CoV-2-containing medium was irradiated with mercury lamp (254 nm) at room temperature for the indicated time periods. The virus-containing medium was 10-fold serially diluted and added to Vero E6 cells for 4-day incubation. Cells were then fixed, stained, and scored for infection. TCID₅₀ was calculated by Reed and Muench Method (1938).

Table 1. Log-reduction value of UV light devices.

Exposure Time	Log-Reduction Value (LRV)			
	5 s	10 s	20 s	40 s
UVC LED (275 nm)	4.23	>6	>6	>6
Mercury lamp (254 nm)	1.17	3.34	>6	>6
Excimer lamp (222 nm)	0.33	0.6	1.83	1.33

For the excimer lamp (222 nm) disinfection test, the untreated SARS-CoV-2 titer ($10^{6.83}$ TCID₅₀/mL) was reduced to $10^{6.5}$, $10^{6.23}$, 10^5 , and $10^{5.5}$ TCID₅₀/mL after exposure to an excimer lamp for 5, 10, 20, and 40 s, respectively (Figure 3). The LRVs were 0.33, 0.6, 1.83 and 1.33 for 5, 10, 20 and 40 s of irradiation, respectively (Table 1).

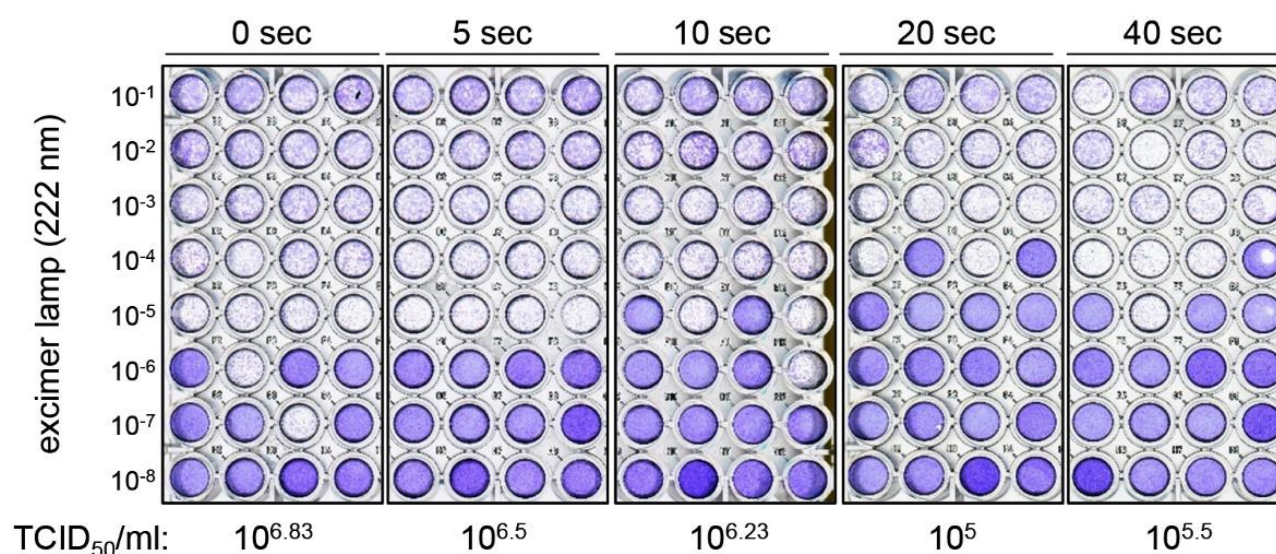


Figure 3. Disinfection efficacy of excimer lamp (222 nm) against SARS-CoV-2 was determined by tissue culture infection dose. SARS-CoV-2-containing medium was irradiated with excimer lamp (222 nm) at room temperature for the indicated time periods. The virus-containing medium was 10-fold serially diluted and added to Vero E6 cells for 4-day incubation. Cells were then fixed, stained, and scored for infection. TCID₅₀ was calculated by Reed and Muench Method (1938).

To confirm the effects of irradiation with UV light devices, we also used an immunofluorescent assay to detect the SARS-CoV-2 N protein expression. As shown in Figure 4, SARS-CoV-2 N protein expression was greatly reduced by exposure to UVC LEDs (275 nm) and the mercury lamp (254 nm), but not much by the excimer lamp (222 nm).

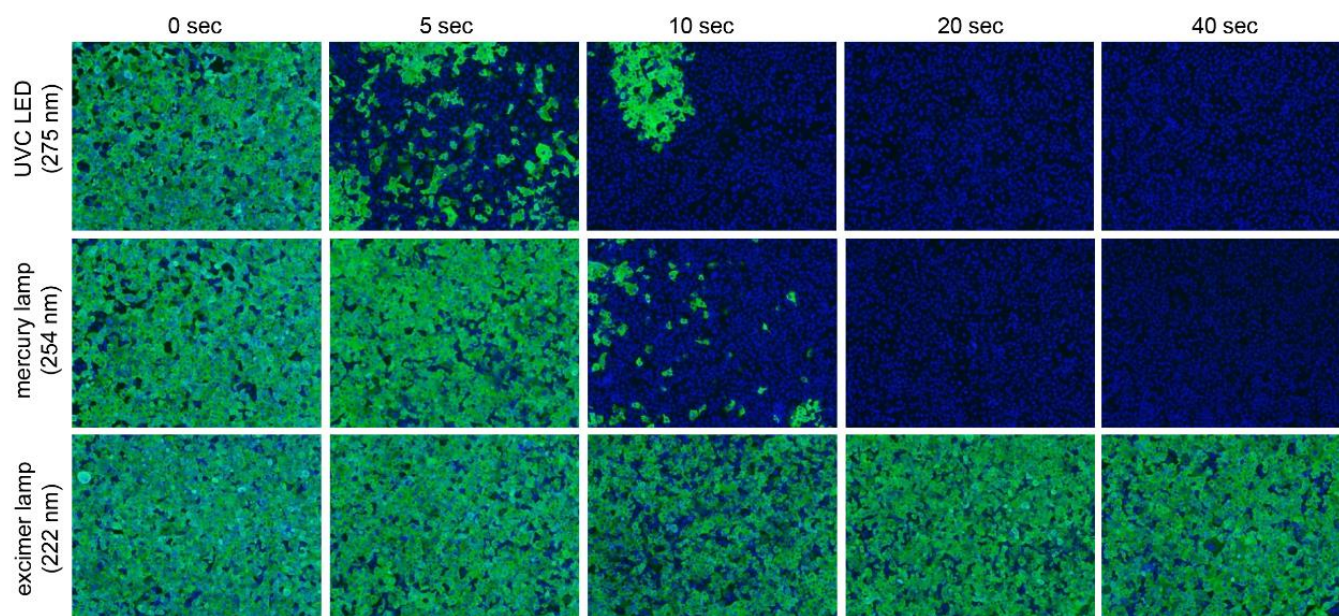


Figure 4. Disinfection efficacy of UVC light devices was determined by immunofluorescent assay. The irradiated samples were added to Vero E6 cells for 1 day incubation. Cells were fixed and immunostained with anti-SARS-CoV-2 N protein antibody and goat anti-human IgG-Alexa Fluor 488 (green). Cell nucleus was stained with DAPI (blue).

4. Discussion

The disinfection effect of UV light on bacteria and viruses largely depends on the wavelength. UVC with the shortest wavelength can provide the greatest performance within the UV range and is currently used to disinfect surfaces, equipment, operating

rooms, and personal protective equipment (PPE) in healthcare settings [18]. UVC LEDs around 254 to 265 nm has been shown to provide the highest disinfection effect within the UVC range [19]. In this study, we further found that UVC LEDs (275 nm) exhibited a superior disinfection ability against SARS-CoV-2 compared to mercury lamps (254 nm) and excimer lamps (222 nm).

The disinfection effect is determined by the cumulative light amount of the ultraviolet light. The cumulative light amount is determined by multiplying the intensity of light and the intensity time. In general, the higher the UVC light intensity, the higher the disinfection effect. Moreover, the longer the irradiation time, the greater the disinfection effect. In addition, the disinfection ability also depends on the wavelength of UVC light. The peak effectiveness at 275 nm is due to the fact that this wavelength is the peak wavelength for RNA/DNA absorption, and hence it can disturb the protein structures effectively. This structural modification will lead to the disintegration of its protein functionality and will hence inactivate the virus. Thus, the UVC light (275 nm) has the strongest disinfection impact even with a lower intensity ($79.8 \mu\text{W}/\text{cm}^2$) and is better than the mercury lamps (254 nm) with a higher intensity ($850 \mu\text{W}/\text{cm}^2$).

Nicola et al. demonstrated the inactivation of SARS-CoV-2 using UVC 275 nm LEDs, showing that there is 99.9% inactivation after 1 min of treatment with a dose of $83.1 \text{ J}/\text{m}^2$ and a minimum irradiance of $1.385 \text{ W}/\text{m}^2$ [20]. In our study, a UVC LED with a lesser irradiation intensity of $79.8 \mu\text{W}/\text{cm}^2$ showed better effectiveness by inactivating SARS-CoV-2 with a reduction of more than 99.99% after only 5 s of irradiation. There is an even greater reduction of more than 99.9999% after 10 s of irradiation, indicating a better effectiveness than the previously reported values. Mara et al. demonstrated the effect of UVC light with a 254 nm wavelength on SARS-CoV-2 inactivation, showing that a very small dose of less than $4 \text{ mJ}/\text{cm}^2$ is enough to achieve full inactivation of the virus [21]. In addition, even at a higher virus input concentration of 1000 MOI, viral replication is totally inactivated with a dose $\geq 16.9 \text{ mJ}/\text{cm}^2$. However, their exposure time is quite long in terms of hours. In our study, we found that there is more than a 99.9999% reduction in the virus count even after 20 s of exposure using a mercury lamp at 254 nm, which is more effective than the study mentioned above.

There are some challenges for UVC disinfection lighting. UVC lamps used for disinfection may cause health and safety issues depending on the wavelength, dose, and duration of radiation exposure. The risk increases if the device is improperly placed or handled by unskilled individuals. Direct UVC radiation exposure to the skin and eyes from some UVC lamps can result in painful eye injury and burn-like skin reactions [22]. Since some UVC lamps contain mercury, which is toxic even in small amounts, considerable caution is required when cleaning and discarding a broken lamp. It is important to mention that if a virus or bacterium is not directly exposed to UVC, it will not be inactivated. For example, if the virus or bacteria is covered in dust or dirt, lodged in a porous surface, or on the underside of a surface, it will not be inactivated.

It is evident that SARS-CoV-2 is mostly transmitted by contacting infected people at close range through inhalation of a “droplet” or “aerosol” [23]. Hence, it is necessary to have improved indoor air quality through proper ventilation and safer indoor environments to protect people from the infection. UVC can be used in disinfection surfaces and indoor spaces as ultraviolet light has been increasingly used in applications outside of medical purposes. For years, hospitals have used UVC light-emitting robots to disinfect patient rooms and operating rooms, as well as medical equipment or instruments [24]. UVC LEDs can also be used to disinfect vehicles such as cars, trains, and aircrafts in a way that robots or human-controlled machines with UVC emission can move through the vehicles to disinfect the surfaces where light can reach. It is also possible to use UVC LEDs to disinfect air in indoor spaces like schools, restaurants, shops, and even housing spaces where UVC LEDs can be installed in air flow systems overhead and aimed at the ceiling to disinfect air as it circulates [25,26]. Similarly, UVC LED light sources can also be installed in heating, ventilation, and air conditioning (HVAC) systems to disinfect air as it

flows via ductwork. Researchers and industries are developing novel UVC light disinfection technologies such as the automation of the disinfection process using robots [27]. Novel UVC air and surface treatment technologies will provide new tools to control the current and future pandemics.

5. Conclusions

In this study, we compared the disinfection effectiveness of three UVC light devices: a UVC LED (275 nm), a mercury lamp (245 nm), and an excimer lamp (222 nm) against SARS-CoV-2 based on the cell-based infection assay. TCID₅₀ and LRV were calculated as a measure of effectiveness for the indicated light devices. The UVC LED (275 nm) was more effective in disinfecting SARS-CoV-2 than the mercury lamp (254 nm), while the excimer lamp (222 nm) was found to have a negligible effect on SARS-CoV-2.

Table 2. Radiation ($\mu\text{J}/\text{cm}^2$) of UV light devices.

Exposure Time	Radiation ($\mu\text{J}/\text{cm}^2$)			
	5 s	10 s	20 s	40 s
UVC LED (275 nm)	399	798	1596	3192
Mercury lamp (254 nm)	4250	8500	17,000	34,000
Excimer lamp (222 nm)	35	70	140	280

Author Contributions: J.-J.L.: C.-C.L., C.-S.C., C.-Y.L. and S.-Y.C. designed and performed the anti-viral experiments. S.-B.H., Y.-F.Y. and K.-M.L. designed the experiments, developed the U.V.C. devices, and executed the reliability test of the devices. K.J.S., H.C.K., J.-J.L. and Y.-L.L. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest:

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