Utilization of Ultraviolet C Light-Emitting Diodes for the Deactivation of SARS-CoV-2 in Human Breath

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Abstract— Ultraviolet light has a long track record when it comes to use for deactivating harmful microorganisms. With recent developments in LED technology in terms of generating electromagnetic waves from this specific spectral range, new applications have emerged. In this study, the possibility of constructing an effective miniature UV barrier for deactivating the SARS-CoV-2 virus in both exhaled and inhaled air is investigated. Our findings demonstrate that utilizing commonly available UVC diodes operating at the wavelength of 275 nm makes it possible to attain an adequate level of deactivation that fulfills the standards specified for commercial devices.

The challenge of controlling and limiting the multiplication of viruses and bacteria has accompanied humanity in various aspects for a long time, leading to the development of numerous disinfection methods. When selecting an appropriate method for a specific application, many factors must be considered, particularly the medium in which the pathogens will be targeted. One of the commonly used non-invasive disinfection methods is the application of bactericidal ultraviolet (UV) radiation [1]. This method, primarily employed mainly to reduce the number of microorganisms in the air, can be successfully utilized in all other environments, including application for surface or object disinfection [2]. Despite the long-standing practical application, continuous technological development and increased demand for epidemiological protection have renewed interest in UV light disinfection and virus deactivation.

With the advent of the COVID-19 pandemic, which disrupted the established order of life, there has been an increased demand for new, innovative methods to combat the spread of viruses without limiting human interactions. When considering virus deactivation with UV radiation, the traditional approach is associated with low-pressure lamps capable of providing light from the required spectral range. The significant disadvantages of such light sources, including their bulky size and the hazardous mercury content (possessing severe health risks and environmental concerns), made it necessary to search for effective and safer alternatives. Fortunately, the development of semiconductor technology has led to the introduction of light-emitting diodes (LEDs), which offer, among others, reliable and efficient sources of UVC radiation. These LEDs are capable of delivering the necessary optical power density for the effective deactivation of microorganisms while at the same time being cost-effective and environmentally friendly [3]. Consequently, their application makes the size of the light source no longer an insurmountable obstacle, enabling the construction of compact solutions [4–5].

When constructing practical devices and systems, one has to remember that the principal aspect of reducing the probability of infection is to prevent the virus from reaching another individual. One of the most interesting solutions to impede the spread of pathogens is the implementation of UV barriers. These barriers inhibit the transmission of microorganisms through the illumination of a designated section of a room from multiple angles, effectively dividing the space into two parts without physically obstructing airflow [6]. Utilizing the same operational principle for mobile personal devices, this method can be adapted to capture viruses in exhaled breath. A system based on this principle must be sufficiently small to be integrated into a mask and ensure that all inhaled and exhaled air passes through a miniature barrier.

To verify whether it is possible to construct a practical implementation of the concept mentioned above using currently available UVC LEDs, a measurement system to mimic the airflow through such a setup has been designed, built, and tested (Fig. 1). For this purpose, a simple model of a miniature UV barrier, utilizing 12 LEDs, each with an optical power density of 0.247 mW/cm² and a wavelength of 275 nm, has been constructed. The maximum current intensity in the circuit with LEDs was about 350mA at a voltage of about 70V. The UVC light source proposed in the experiment was optimal in terms of the power of the LED light and has been chosen for the illuminated tube under the tests.

Such particular diodes were selected based on (i) the exposure time, which has to be as short as possible due to the aerosol motion, and (ii) the wavelength, offering good deactivation capability while being less harmful to humans and thus allowing for higher doses to be applicated [8–9]. To simulate breathing, the KT-NEB Family compressor inhaler (by Kardio-Test Medical Company) has been used, not only providing the appropriate airflow speed but also converting the virus suspension into an aerosol. To maintain the sterility of the system, the virus passed through a quartz tube with a 90% transmission efficiency, ultimately settling in a vial. The radiation dose of UVC

light (expressed by the optical power density) received by the virus particles, calculated based on the system components, was 5.24 mJ/cm². Comparing this value with UV radiation doses used for the SARS-CoV-2 virus deactivation [8–9], the reduction in titers achieved by the proposed system should be approximately 85%. For safety reasons, instead of testing the system on the SARS-CoV-2 virus, its close relative, namely, the human betacoronavirus HCoV-OC43 from the ATCC collection, has been used. Notably, the effects of infection with this virus are milder for humans, and its response to UV radiation is similar.

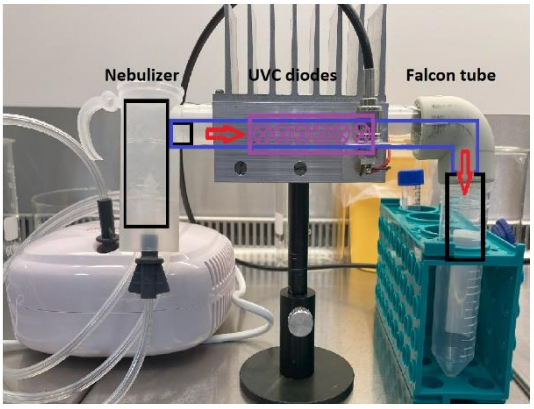

Fig. 1. Photo and scheme of the proposed measurement system to mimic the airflow through a miniature UVC barrier to be potentially applied to deactivate the SARS-CoV-2 virus in human breath. Please note that only half of the number of diodes is shown in the diagram (the second half is located on the other side of the measuring system).

The efficiency of the miniature UV barrier was evaluated by quantifying the variation in virus particle count between irradiated and non-irradiated samples. To achieve this, the virus suspension, after passing through the constructed system, was diluted to obtain various concentrations of the supernatant with the maintenance medium. Subsequently, infections were performed on cultured cell monolayers in 96-well plates, ensuring similar cell numbers in each well.

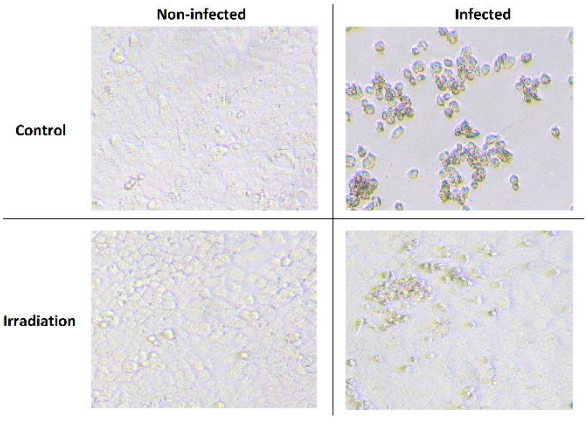

Fig. 2. Microscopic images of cell colonies treated with UVC-irradiated and non-irradiated human betacoronavirus HCoV-OC43.

The reduction titer analysis was conducted using three different methods. The primary method involved evaluating the occurrence of the cytopathic effect, as shown in Fig. 2, using Kärber's formula [10]:

$$log\ TCID_{50} = L - d\ (S - 0.5), \qquad (1)$$

where L - represents the logarithm of the smallest dilution utilized in the experiment, d - denotes the difference in the logarithm of dilutions, S - signifies the sum of the proportions of "positive" results (occurrence of the cytopathic effect), and $TCID_{50}$ indicates the infectious dose for 50% of the cell culture. The second method involved quantifying the number of virus particles based on UV absorption. Following an 8-day incubation period, the HCoV-OC43 virus was subsequently mixed with lysis buffer in ratios of 1:3, 1:5, 1:10, 1:50, and 1:100 (buffer volume: virus volume). The samples were incubated at 95°C for 15 minutes, centrifuged briefly, and preserved on ice. The viral particles (VPs) in the solution were assessed concerning RNA content, which was quantified utilizing a Spark microplate reader (Tecan, Männedorf, Switzerland). The UV absorbance was recorded at both wavelengths of 260 nm to determine the RNA content of HCoV-OC43 and at 280 nm to measure protein content. Additionally, RNA purity was evaluated with a 260 nm/280 nm ratio of 2.0. The concentration of viral particles was calculated following the methodology described by Maizel [11]. The extinction coefficient was 1.1×10^{12} viral particles per OD 260 unit. The viral particle concentration was determined according to the following equation:

$$\mathrm{VP} = \mathrm{A260} \cdot \text{dilution factor} \cdot \frac{1.1 \cdot 10^{12}}{\mathrm{mL}}. \qquad (2)$$

The reduction in titer for both methods was calculated based on the obtained values using the specified equation:

$$\mathrm{ITR} = \left(1 - \frac{1}{10^{\log_{10}\frac{N_0}{N_t}}}\right) \cdot 100\%, \qquad (3)$$

where N_t is the titer of the UVC-irradiated sample and N_0 is the titer of the sample without irradiation [12].

Table 1. The average titer reduction was obtained using Kärber's Titer and UV spectrophotometry, respectively.

Method	Average titer reduction [%]
Kärber's Titer	92.03
UV Spectrophotometry	88.95

The reductions in viral titer calculated for both methods were approximately 90% (see Tab. 2), which exceeds the expected values based on the system parameters. This discrepancy suggests that the actual dose of UV exposure to the virus was higher than initially estimated. This overestimation could be attributed to the approximations made during the dose calculation process.

The relative quantification of the HCoV-OC43 genome was conducted utilizing a one-step real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR). This process involved RNA extraction from supernatants, which was performed using the Total RNA Maxi kit (A&A Biotechnology, Gdansk, Poland). Real-time one-step RT–qPCR was performed using the Express One-Step Superscript qRT–PCR Kit (Invitrogen, Carlsbad, CA, USA). The RT-qPCR conditions were as follows: following the activation of the polymerase (15 min at 50°C), there were 40 cycles of amplification (15 s at 95°C, 30 s at 57.6°C), and finally, a melt curve was generated (65–95°C increment 0.5°C). Relative quantification (RQ) for each RNA sample was calculated using the following equation:

$$RQ = \text{Cq control} - \text{Cq sample}, \qquad (4)$$

where Cq is the quantification cycle of untreated control virus and irradiated virus sample. Upon thoroughly analyzing the melting curve, it was determined that the samples exclusively contained RNA from the HCoV-OC43 virus. No extraneous RNA sequences were detected, indicating the specificity and purity of the viral RNA present in the samples.

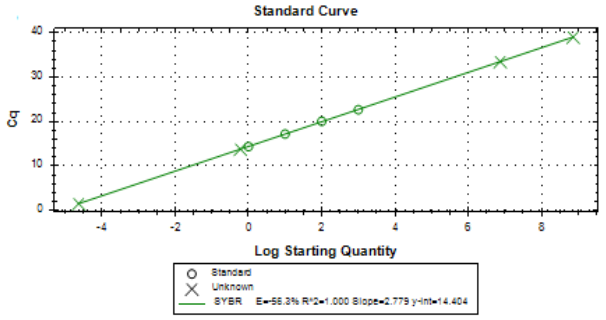

Fig. 3. Graph of the calibration curve generated for the OC-43 control using primers.

In the case of RT-qPCR, based on the calibration curve generated by the software (Fig. 2), a significant difference in the amount of RNA was observed between untreated and UVC-treated samples of the same dilution. The quantification cycle occurred significantly later, indicating the effectiveness of the applied system. The obtained results exhibited high measurement accuracy, as the coefficient of determination for the calibration curve was 1. The average relative quantification for the measurements was –15.91.

Based on the results, it can be concluded that we have successfully constructed a functional miniature version of a UV barrier, which effectively deactivates the SARS-CoV-2 virus in the air during breathing. Our system achieves a reduction rate of approximately 90%, thereby meeting the essential requirement for UVGI air systems, which is a D90 value (i.e., 90% reduction in the number of microorganisms). Considering the reduced UV dose received due to less than 100% transmission for the quartz glass and a higher airflow rate than human breathing at rest, the obtained result is satisfactory and allows for effective use of such a system in practical applications.

The only drawback of the proposed solution may be the number of diodes used, which could make a system impossible to be constructed when using the traditional mask in which the UV barrier could replace the filter without access to more powerful UVC LEDs. The latter may reduce the number of diodes needed to achieve the same virus reduction rate, allowing for further system miniaturization. Anyhow, it may be stated that using ultraviolet light to deactivate viruses in breath is an effective method to prevent virus spread, and the currently available technology allows us to construct proposed devices based on UV barriers.

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