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Investigation of Antiviral Effect of Far-UVC Microplasma Lamp against Influenza A Virus (H9N2)

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Abstract

Influenza A virus is one of the most serious diseases in the world. Therefore, it is necessary to find an effective and safe method to prevent the spread of the disease. A far-UVC at 222nm is considered safe and effective for viral and bacterial treatment. In this study, virucidal effects and the safety status of far-UVC microplasma were evaluated *in vitro* against influenza A virus H9N2 0130 strain. The results (from $TCID_{50}$ and real-time PCR) indicated that a far-UVC inhibited influenza A virus depending on dosage. A far-UVC eliminated 99.99% of the virus at doses of 44 and 56 mJ/cm^2 in clarified and un-clarified solutions, respectively. Moreover, a far-UVC 222 nm did not have any harmful effects in MDCK cell at dose 78 mJ/cm^2. Our study provided useful information in a far-UVC application against influenza A virus.

Introduction

Influenza A virus (IAV), an enveloped virus with segmented, negative single-strand RNA linear genome, is one of the most serious pathogens in the world, causing significantly negative impacts on economy and human-animal health (Epstein & Price, 2009). Based on its surface antigens, IAV was classified into different subtypes related to the antigenic characteristics of hemagglutinin (HA) and neuraminidase [1]. To date, 18 types of HA (H1 - H18) and 11 type of NA (N1 - N11) were identified [2], among which the last two HA and NA subtypes tended to be specific to bats [3]. Subtypes H1N1 and H3N2 currently spread throughout the human population [4-7]. Similarly, H5, H7, and H9 still cause serious problem in poultry production as evidenced by high mortality rate and loss of egg production.

Due to their rapid rate of contagion, it is difficult to effectively control IAV and other air-borne diseases. Disinfectant agents might be harmful for human health, possibly causing eye and skin irritation, and may result in damage to the equipment surfaces such as discoloration in textiles due to corrosive metals [8]. Ultraviolet light at wavelength of 254 nm or above, which is also widely applied to prevent diseases, may cause skin cancer and cataracts [9]. Recently, the application of far-UVC light at wavelength range of 200 - 230 nm as a potential disinfection method has been the interest of many studies [10]. This type of UV was demonstrated to effectively inactivate a numerous of pathogens including bacteria and viruses [5,10]. This type of far-UVC is also considered safe for humans [8]. However, not many studies focused on controlling the spread of IAV. In this study, we investigated the virucidal effects against H9N2 as an IAV subtype model using a microplasma far-UVC lamp, primarily emitting a wavelength of 222 nm.

Material & Methods

A microplasma lamp (UV222050 x 050, Eden Park Illumination, Inc., Champain, IL, USA) with the emission wavelength of 222 nm was applied and the UV irradiation fixture and setup were designed and prepared (NANOCMS Co., Ltd., Cheonan, Korea) to have an adjustable distance between the target sample surface and light source. The UV exposure conditions were well described in the previous study [9]. IAV serotype H9N2 01310 vaccine strain and MDCK cell line were kindly provided by Professor Kang Suk Choi (Avian laboratory, College of Veterinary Medicine, Seoul Nation University). Virus solution was spread in Petri dishes (60 mm) and the irradiation time was varied from 10 seconds (1.3 mJ/cm^2) to 10 minutes (78 mJ/cm^2). Treated virus and non-treated control were serially diluted in maintain media (DMEM plus 1 μg/ml TPCK-treated trypsin and 1% NEAA) and inoculated in to MDCK cell cultured in 96-wells plate. After 1 hour of adsorption, the cells were carefully washed three times, replaced by 100 μl of fresh maintain media and incubated at 37°C, 5% CO_2 for 5 days. The cells were observed daily to detect the presence of cytopathic effects and $TCID_{50}$ was calculated using the Reed and Muench method [11,12]. Each condition was tested three times.

The presence of genetic trace of IAV was also examined by quantitative RT-PCR. Viral RNA was extracted from treated solution using RNA extraction kit (Intron Biotech, Korea) according to the manufacturer's protocol. RNA was converted to cDNA using SuperScript III First-strand synthesis kit (Invitrogen, USA). Real-time PCR was performed using Maxima Sybr green/Rox qPCR master mix (ThermoFisher, USA) using specific primers (Table 1).

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Table 1: Real-time PCR primers used in the study.

Primer name	Sequence	Size (bp)
M-qPCR-F	TTGCACTTGATATTGTGGAT	119
M-qPCR-R	TCTTCCCTCATAGACTCAGG	

Cytotoxic analysis of far UVC irradiation was performed using MTT assay. In brief, 5 x 10^4 MDCK cell were seeded in to each well of the 96 wells plate and incubated at 37°C, 5% CO_2 overnight. Cell was irradiated with far UVC light for 10 minutes. The viability of cells was evaluated using CyQUANT™ MTT Cell Viability Assay (Invitrogen, USA) according to manufacturer's instruction.

Statistical analysis was performed using GraphPad Prism 8.0 (GraphPad Software Inc., USA). Virus titer in each treated condition was compared using one-way ANOVA and Turkey analysis. The inhibition growth curve was calculated using nonlinear regression curve analysis.

Results & Discussion

First, we investigated the effect of far-UVC microplasma on clarified virus. The results indicated that, far-UVC (222nm) inhibited AIV serotype H9N2 01310 vaccine strain in a dose-dependent manner. Specifically, a dose of 2.6 mJ/cm^2 significantly reduced the viral titer when compared to the untreated condition (Figure 1A). Additionally, 78 mJ/cm^2 exposure doses (corresponding to 10 minutes of treatment) inhibited almost all viruses in the experimental condition (Figure 1A). Moreover, to answer the question about the effect of cell debris on virucidal activity of far-UVC, we performed a similar experiment with un-clarified virus solution. Similar trend of virus inhibition was also noticed in this experiment (Figure 1B). 2.6 mJ/cm^2 irradiated dose decreased the virus titer by approximately 0.8 log_{10} $TCID_{50}$ while UVC irradiated at 78 mJ/cm^2 caused a reduction of virus titer to 1.8 log_{10} $TCID_{50}$ (Figure 1B). These results were supported by the reduction of viral RNA trace (Figure 1C).

The effective irradiation doses, which was defined as the treatment condition that reduced virus by 4 log_{10} $TCID_{50}$ was calculated based on the dose-inhibition curve as described in the method section. The result indicated that the effective irradiation doses were approximate 44 and 56 mJ/cm^2 in clarified and un-clarified solution, respectively. Therefore, far-UVC microplasma irradiation was slightly more effective against clarified virus than cell-debris containing fluid (Figure 1D).

Previous study indicated that irradiation dosage at 7.8 mJ/cm^2 eliminated almost all SARS-CoV-2 in solution (Jung et al., 2021). Moreover, Buonanno, Welch, Shuryak, and Brenner (2020) demonstrated that lose dose at 1.7 and 1.2 mJ/cm^2 can remove 99.9% of alpha HCoV-229E and beta HCoV-OC43 in aerosol [3]. For IAV, 222 nm UVC at 2mJ/cm^2 can inactivate more than 95% of aerosolized H1N1 [14-17]. However, in our study, irradiated doses at approximately 23 mJ/cm^2 and 33 mJ/cm^2 were necessary to inactivated 99.9% H9N2 virus in clarified and unclarified solutions, respectively. The higher effective dose in this study might be due to its wavelength that penetrated less into the liquid solution. In this study, comparing with the clarified sample, cell-debrid containing sample need a higher dose of irradiation. This result could be explained by the fact that cell-debrid might absorb the UV energy, resulted in decrease the virucidal efficiency. Ma et al. also suggested the effect

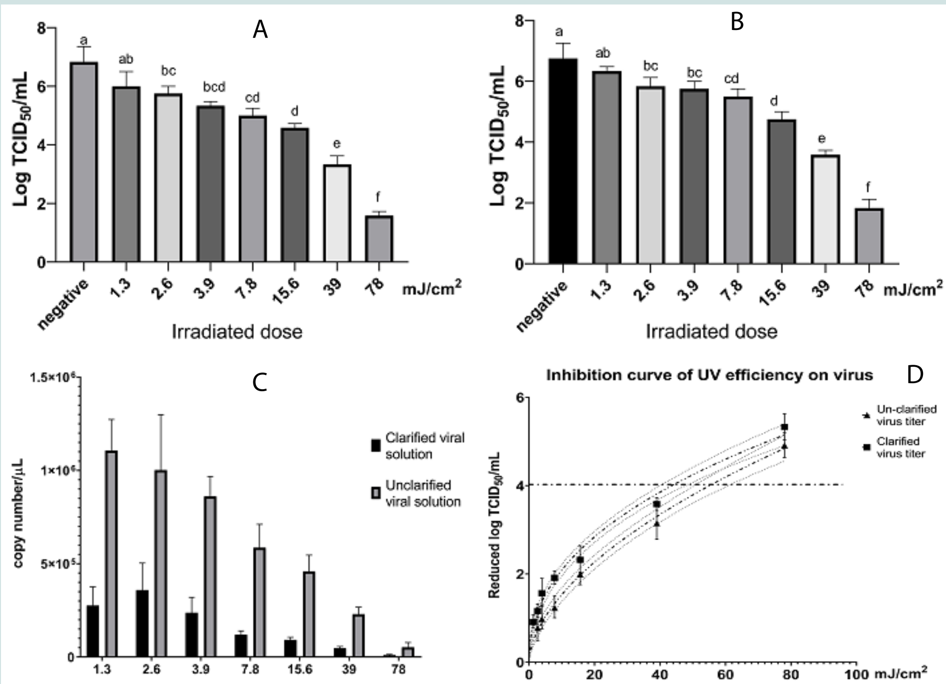

Figure 1: A) The far-UVC dose response of IAV serotype H9N2 01310 vaccine strain in clarified solution. B: Virus titer in unclarified solution after treatment at different time-point. C: The copy number (copies/µl) of viral genetic traces after treatment at different doses. D: Inhibition curve of far-UVC against virus IAV in clarified and un-clarified samples. Letters a, b, c, d, e and f indicate UV expose condition showing significant differences ($P < 0.05$) compared to the negative control. Different letters mean that there was a significant difference ($P < 0.05$) between two groups.

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of media component on the virus sensitivity to UV exposure [10]. Nevertheless, this study provided useful evidence of antiviral activity of far-UVC light in aqueous solution.

In our study, 10 minutes treatment effectively reduced the infectivity of IAV serotype H9N2 01310 vaccine strain. Therefore, we continuously examined the cytotoxic effect of this condition under *in-vitro* experimental conditions. MTT assay revealed that 78 mJ/cm^2 irradiated dose did not cause harmful interference against MDCK cell line under experimental condition (Figure 2). A far-UVC 222 nm wavelength light was considered safe for humans. In detail, long-term exposure to far-UVC microplasma at 222 nm wavelength could not induce cancer in the sensitive model experiment [18,19]. Similarly, Fukui et al. (2020) indicated that UVC with wavelength of 222 nm at a dose of 500 mJ/cm^2 only slightly induced DNA damage in skin after treatment [7]. In our study, there were no differences in cell survival in exposed experimental and non-exposed control, indicating the safety of 222 nm far- UVC at a dose of 78 mJ/cm^2 *in vitro*.

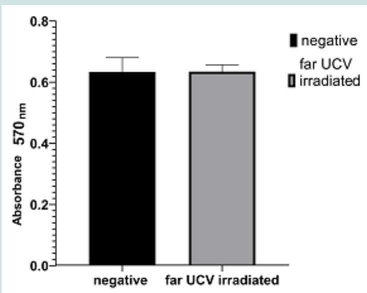

Figure 2: Cell viability evaluation was performed using MTT assay. The histogram indicated that there were no different in cell survival between treated sample and non-treated control. Data shown as mean absorbance values (A570 nm) of triplicate wells and error bars represent standard deviation (SD).

Conclusion

In conclusion, this study demonstrated that far UVC microplasma irradiation effectively removes the infectivity of influenza virus without harming the cell. Our results suggested the effectiveness and safety of far UVC microplasma irradiated dose.

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