1   # Far-UVC light: A new tool to control the spread of airborne-mediated microbial

2   diseases

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10

11   **Abstract**

12   Airborne-mediated microbial diseases such as influenza and tuberculosis represent major public

13   health challenges. A direct approach to prevent airborne transmission is inactivation of airborne

14   pathogens, and the airborne antimicrobial potential of UVC ultraviolet light has long been established;

15   however, its widespread use in public settings is limited because conventional UVC light sources are

16   both carcinogenic and cataractogenic. By contrast, we have previously shown that far-UVC light (207-

17   222 nm) efficiently kills bacteria without harm to exposed mammalian skin. This is because, due to its

18   strong absorbance in biological materials, far-UVC light cannot penetrate even the outer (non living)

19   layers of human skin or eye; however, because bacteria and viruses are of micrometer or smaller

20   dimensions, far-UVC can penetrate and inactivate them. We show for the first time that far-UVC

21 efficiently kills airborne aerosolized viruses, a very low dose of 2 mJ/cm^2 of 222-nm light inactivating

22 >95% of aerosolized H1N1 influenza virus. Continuous very low dose-rate far-UVC light in indoor

23 public locations is a promising, safe and inexpensive tool to reduce the spread of airborne-mediated

24 microbial diseases.

25

26   Airborne-mediated microbial diseases represent one of the major challenges to worldwide public

27   health[1]. Common examples are influenza[2], appearing in seasonal[3] and pandemic[4] forms, and bacterially-

28   based airborne-mediated diseases such as tuberculosis[5], increasingly emerging in multi-drug resistant

29   form.

30   A direct approach to prevent the transmission of airborne-mediated disease is inactivation of the

31   corresponding airborne pathogens, and in fact the airborne antimicrobial efficacy of ultraviolet (UV)

32   light has long been established[6-8]. Germicidal UV light can also efficiently kill both drug-sensitive and

33   multi-drug-resistant bacteria[9], as well differing strains of viruses[10]. However, the widespread use of

34   germicidal ultraviolet light in public settings has been very limited because conventional UVC light

35   sources are a human health hazard, being both carcinogenic and cataractogenic[11,12].

36   By contrast, we have earlier shown that far-UVC light generated by filtered excimer lamps

37   emitting in the 207 to 222 nm wavelength range, efficiently kills drug-resistant bacteria, without

38   apparent harm to exposed mammalian skin[13-15]. The biophysical reason is that, due to its strong

39   absorbance in biological materials, far-UVC light does not have sufficient range to penetrate through

40   even the outer layer (stratum corneum) on the surface of human skin, nor the outer tear layer on the

41   outer surface of the eye, neither of which contain living cells; however, because bacteria and viruses are

42   typically of micron or smaller dimensions, far-UVC light can still efficiently traverse and inactivate

43   them[13-15].

44   The earlier studies on the germicidal efficacy of far UVC light[13,15-18] were performed exposing

45   bacteria irradiated on a surface or in suspension. In that a major pathway for the spread of influenza A is

46   aerosol transmission[3], we investigate for the first time the efficacy of far-UVC 222-nm light for

47   inactivating airborne viruses carried by aerosols – with the goal of providing a potentially safe

48   alternative to conventional 254-nm germicidal lamps to inactivate airborne microbes.

Results

Virus inactivation. Fig. 1 shows representative fluorescent 40x images of mammalian epithelial cells incubated with airborne viruses that had been exposed in aerosolized form to far-UVC doses (0, 0.8, 1.3 or 2.0 mJ/cm^2) generated by filtered 222-nm excimer lamps. Blue fluorescence was used to identify the total number of cells in a particular field of view, while green fluorescence indicated the integration of live influenza A (H1N1) viruses into the cells. Results from the zero-dose control studies (Fig. 1, top left) confirmed that the aerosol irradiation chamber efficiently transmitting the aerosolized viruses through the system, after which the live virus efficiently infected the test mammalian epithelial cells.

Fig. 2 shows the surviving fraction, as a function of the incident 222-nm far-UVC dose, of exposed H1N1 aerosolized viruses, as measured by the number of focus forming units in incubated epithelial cells relative to unexposed controls. Linear regressions (see below) showed that the survival results followed a classical exponential UV disinfection model with rate constant k=1.8 cm^2/mJ (95% confidence intervals 1.5-2.1 cm^2/mJ). The overall model fit was good, with a coefficient of determination, R^2= 0.95, which suggests that most of the variability in virus survival was explained by the exponential model. The rate constant of 1.8 cm^2/mJ corresponds to an inactivation cross-section (dose required to kill 95% of the exposed viruses) of D_{95} = 1.6 mJ/cm^2 (95% confidence intervals 1.4-1.9 mJ/cm^2).

Discussion

We have developed an approach to UV-based sterilization using single-wavelength far-UVC light generated by filtered excilamps, which selectively inactivate microorganisms, but does not produce biological damage to exposed mammalian cells and tissues[13-15]. The approach is based on biophysical

4

71 principles in that far-UVC light can traverse and therefore kill bacteria and viruses which are typically

72 micrometer dimensions or smaller, whereas due to its strong absorbance in biological materials, far-

73 UVC light cannot penetrate even the outer dead-cell layers of human skin, nor the outer tear layer on the

74 surface of the eye.

75   Here we applied this approach to test the efficacy of the 222-nm far-UVC light to kill influenza

76 A virus (H1N1) carried by aerosols in a benchtop aerosol UV irradiation chamber, which generated

77 aerosol droplets of sizes similar to those generated by human coughing and breathing. Aerosolized

78 viruses flowing through the irradiation chamber were exposed to UVC emitting lamps placed in front of

79 the chamber window.

80   As shown in Fig. 2, killing of influenza A virus (H1N1) by 222-nm far-UVC light follows a

81 typical exponential disinfection model, with an inactivation cross-section of $D_{95} = 1.6$ mJ/cm^2 (95% CI:

82 1.4-1.9). For comparison, using a similar experimental arrangement, but using a conventional 254 nm

83 germicidal UVC lamp, McDevitt *et al.*[19] found a D_{95} value of 1.1 mJ/cm^2 (95% CI: 1.0-1.2) for H1N1

84 virus. Thus as we[13,15] and others [16-18] reported in earlier studies for bacterial inactivation, 222-nm far-

85 UVC light and 254-nm broad-spectrum germicidal light are quite similar in their efficiencies for viral

86 inactivation, the comparatively small differences presumably reflecting differences in nucleic acid

87 absorbance. However as discussed above, based on biophysical considerations and in contrast to the

88 known human health safety issues associated with conventional germicidal 254-nm broad-spectrum

89 UVC light, far-UVC light does not appear to be cytotoxic to exposed human cells and tissues *in vitro* or

90 *in vivo*[13-15].

91   If these results are confirmed in other scenarios, it follows that the use of overhead low-level far-

92 UVC light in public locations may represent a safe and efficient methodology for limiting the

93 transmission and spread of airborne-mediated microbial diseases such as influenza and tuberculosis. In

5

94   fact the potential use of ultraviolet light for airborne disinfection is by no means new, and was first

95   demonstrated more than 80 years ago[8,20]. As applied more recently, airborne ultraviolet germicidal

96   irradiation (UVGI) utilizes conventional germicidal UVC light in the upper part of the room, with

97   louvers to prevent direct exposure of potentially occupied room areas[21]. This results in blocking more

98   than 95% of the UV radiation exiting the UVGI fixture, with substantial decrease in effectiveness[22]. By

99   contrast, use of low-level far-UVC fixtures, which are potentially safe for human exposure, could

100   provide the desired antimicrobial benefits without the accompanying human health concerns of

101   conventional germicidal lamp UVGI.

102   A key advantage of the UVC based approach, which is in clear contrast to vaccination

103   approaches, is that UVC light is likely to be effective against all airborne microbes. For example, while

104   there will almost certainly be variations in UVC inactivation efficiency as different influenza strains

105   appear, they are unlikely to be large[7,10]. Likewise, as multi-drug-resistant variants of bacteria emerge,

106   their UVC inactivation efficiencies are also unlikely to change greatly[9].

107   Finally it is of course by no means the case that all microbes are harmful, and it is well

108   established that the human microbiome is essential to human health[23]. With the exception of a subset of

109   the skin microbiome, all the human microbiome would be entirely shielded from far-UVC light due to

110   its very short range; in fact even within the skin biome only those biota on the skin surface[24] would be

111   potentially affected, which are of course the same biota that are potentially removed by hand

112   sanitizers[25].

113   In conclusion, we have shown for the first time that very low doses of far-UVC light efficiently

114   kill airborne viruses carried by aerosols. For example, a very low dose of 2 mJ/cm^2 of 222-nm light

115   inactivates >95% of airborne H1N1 virus. Our results indicate that far-UVC light is a powerful and

116   inexpensive approach for prevention and reduction of airborne viral infections without the human health

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117   hazards inherent with conventional germicidal UVC lamps. If these results are confirmed in other

118   scenarios, it follows that the use of overhead very low level far-UVC light in public locations may

119   represent a safe and efficient methodology for limiting the transmission and spread of airborne-mediated

120   microbial diseases. Public locations such as hospitals, doctors' offices, schools, airports and airplanes

121   might be considered here. This approach may help limit seasonal influenza epidemics, transmission of

122   tuberculosis, as well as major pandemics.

123

Methods

124   **Methods**

125   **Far-UVC lamps.** We used a bank of three excimer lamps containing a Kr-Cl gas mixture that

126   predominantly emits at 222 nm [26,27]. The exit window of each lamp was covered with a custom bandpass

127   filter designed to remove all but the dominant emission wavelength as previously described[15]. Each

128   bandpass filter (Omega Optical, Brattleboro, VT) had a center wavelength of 222 nm and a full width at

129   half maximum (FWHM) of 25 nm and enables >20% transmission at 222 nm. A UV spectrometer

130   (SPM-002-BT64, Photon Control, BC, Canada) with a sensitivity range between 190 nm and 400 nm

131   was utilized to verify the 222 nm emission spectrum. A deuterium lamp standard with a NIST-traceable

132   spectral irradiance (Newport Model 63945, Irvine, CA) was used to radiometrically calibrate the UV

133   spectrometer.

134   **Far-UVC dosimetry.** Optical power measurements were performed using an 818-UV/DB low-power

135   UV enhanced silicon photodetector with an 843-R optical power meter (Newport, Irvine, CA).

136   Additional dosimetry to determine the uniformity of the UV exposure was performed using far-UVC

137   sensitive film as described in our previous work[28,29]. This film has a high spatial resolution with the

138   ability to resolve features to at least 25 µm, and exhibits a nearly ideal cosine response[30,31].

139   Measurements were taken between experiments therefore allowing placement of sensors inside the

140   chamber.

141   A range of far-UVC exposures, from 3.6 μJ/cm^2 up to 281.6 mJ/cm^2, were used to define a

142   response calibration curve. Films were scanned as 48 bit RGB TIFF images at 150 dpi using an Epson

143   Perfection V700 Photo flatbed scanner (Epson, Japan) and analyzed with radiochromic film analysis

144   software[32] to calculate the total exposure based on measured changes in optical density.

145   Measurements using both a silicon detector and UV sensitive films were combined to compute

146   the total dose received by a particle traversing the exposure window. The three vertically stacked lamps

147   produced a nearly uniform dose distribution along the vertical axis thus every particle passing

148   horizontally through the irradiation chamber received an identical dose. The lamp width (100 mm) was

149   smaller than the width of the irradiation chamber window (260 mm) so the lamp power was higher near

150   the center of the irradiation chamber window compared to the edge. The UV sensitive film indicated a

151   power of approximately 120 μW/cm^2 in the center third of the window and 70 μW/cm^2 for the outer

152   thirds. The silicon detector was used to quantify the reflectivity of the aluminum sheet at approximately

153   15% of the incident power. Combining this data allowed the calculation of the average total dose of 2.0

154   mJ/cm^2 to a particle traversing the window in 20 seconds. Additionally, the silicon detector was used to

155   confirm the attenuation of 222-nm light through a single sheet of plastic film was 65%. The addition of

156   one or two sheets of plastic film between the lamps and the irradiation chamber window yielded average

157   doses of 1.3 mJ/cm^2 and 0.8 mJ/cm^2, respectively.

158   **Benchtop aerosol irradiation chamber.** A one-pass, dynamic aerosol / virus irradiation chamber was

159   constructed in a similar configuration to that used by Ko et al.[33], Lai et al.[34], and McDevitt et al.[19,35]. A

160   schematic overview of the system is shown in Fig. 3. Aerosolized viruses were generated by adding a

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161   virus solution into a high-output extended aerosol respiratory therapy (HEART) nebulizer (Westmed,

162   Tucson, AZ) and operated using a dual-head pump (Thermo Fisher 420-2901-00FK, Waltham, MA)

163   with an input flow rate of 11 L/min. The aerosolized virus flowed into the irradiation chamber where it

164   was mixed with independently controlled inputs of humidified and dried air. Humidified air was

165   produced by bubbling air through water, while dry air was provided by passing air through a desiccant

166   air dryer (X06-02-00, Wilkerson Corp, Richland, MI). Adjusting the ratio of humid and dry air enabled

167   control of the relative humidity (RH) within the irradiation chamber which, along with the nebulizer

168   settings, determined the aerosol particle size distribution. An optimal RH value of 55% resulted in a

169   distribution of aerosol particle sizes similar to the natural distribution from human coughing and

170   breathing, which has been shown to be distributed around approximately 1 μm, with a significant tail of

171   particles less than 1 μm[36-38].

172   After combining the humidity control inputs with the aerosolized virus, input flow was directed

173   through a series of baffles that promoted droplet drying and mixing to produce an even particle

174   distribution [34]. The RH and temperature inside the irradiation chamber were monitored using an Omega

175   RH32 meter (Omega Engineering Inc., Stamford, CT) immediately following the baffles. A Hal

176   Technologies HAL-HPC300 particle sizer (Fontana, CA) was adjoined to the irradiation chamber to

177   allow for sampling of particle sizes throughout operation.

178   During UV exposure, the three 222-nm lamps with filters were stacked vertically and placed 11

179   cm from the irradiation chamber window. The lamps were directed at the 26 cm × 25.6 cm chamber

180   window which was constructed of 254-μm thick UV transparent plastic film (Topas 8007X10, Topas

181   Advanced Polymers, Florence, KY), and which had a transmission of ~65% at 222 nm. The wall of the

182   irradiation chamber opposite the transparent window was constructed with polished aluminum in order

183   to reflect a portion of the UVC light back through the exposure region, therefore increasing the overall

184   exposure dose by having photons pass in both directions. The depth of the irradiation chamber between

185   the window and the aluminum panel was 6.3 cm, creating a total exposure volume of 4.2 L.

186   Flow of the aerosols continues out of the irradiation chamber to a set of three way valves that

187   could be configured to either pass through a bypass channel (used when no sampling was required), or a

188   BioSampler (SKC Inc, Eighty Four, PA) used to collect the virus. The BioSampler uses sonic flow

189   impingement upon a liquid surface to collect aerosols when operated at an air flow of 12.5 L/min.

190   Finally, flow continued out of the system through a final HEPA filter and to a vacuum pump

191   (WP6111560, EMD Millipore, Billerica, MA). The vacuum pump at the end of the system powered flow

192   through the irradiation chamber. The flow rate through the system was governed by the BioSampler.

193   Given the flow rate and the total exposure volume of the irradiation chamber, 4.2 L, a single aerosol

194   droplet passed through the exposure volume in approximately 20 seconds.

195   The entire irradiation chamber was set up inside a certified class II type A2 biosafety cabinet

196   (Labconco, Kansas City, MO). All air inputs and outputs were equipped with HEPA filters (GE

197   Healthcare Bio-Sciences, Pittsburgh, PA) to prevent unwanted contamination from entering the chamber

198   as well as to block any of the virus from releasing into the environment.

199   **Irradiation chamber performance.** The custom irradiation chamber simulated the transmission of

200   aerosolized viruses produced via human coughing and breathing. The chamber operated at a relative

201   humidity of 55% which resulted in a particle size distribution of 87% between 0.3 μm and 0.5 μm, 11%

202   between 0.5 μm and 0.7 μm, and 2% > 0.7 μm. Aerosolized viruses were efficiently transmitted through

203   the system as evidenced from the control (zero exposure) showing clear virus integration (Fig. 1, top

204   left).

205   **Experimental protocol.** The virus solution in the nebulizer consisted of 1 ml of Dulbecco's Modified

206   Eagle's Medium (DMEM, Life Technologies, Grand Island, NY) containing 10^8 focus forming units per

207   ml (FFU/ml) of influenza A virus [A/PR/8/34 (H1N1)], 20 ml of deionized water, and 0.05 ml of Hank's

208   Balanced Salt Solution with calcium and magnesium (HBSS^{++}). The irradiation chamber was operated

209   with aerosolized virus particles flowing through the chamber and the bypass channel for 15 minutes

210   prior to sampling, in order to establish the desired RH value of ~55%. Sample collection initiated by

211   changing air flow from the bypass channel to the BioSampler using the set of three way valves. The

212   BioSampler was initially filled with 20 ml of HBSS^{++} to capture the aerosol. During each sampling time,

213   which lasted for 30 minutes, the inside of the irradiation chamber was exposed to 222 nm far-UVC light

214   through the UVC semi-transparent plastic window. Variation of the far-UVC dose delivered to aerosol

215   particles was achieved by inserting additional UVC semi-transparent plastic films, identical to the

216   material used as the chamber window, between the lamps and the chamber window. The extra plastic

217   films uniformly reduced the power entering the chamber. The three test doses of 0.8, 1.3 and 2.0

218   mJ/cm^2, were achieved by adding two, one, or no additional plastic films, respectively. Zero-dose

219   control studies were conducted with the excimer lamps turned off. Experiments at each dose were

220   repeated in triplicate. After the sampling period was completed the solution from the BioSampler was

221   used for the virus infectivity assay.

222   **Virus infectivity assay.** We measured viral infectivity with a focus forming assay that employs

223   standard fluorescent immunostaining techniques to detect infected host cells and infectious virus

224   particles[39]. Briefly, after running through the irradiation chamber for 30 minutes, 0.5 ml of virus

225   suspension collected from the BioSampler was overlaid on a monolayer of Madin-Darby Canine Kidney

226   (MDCK) epithelial cells routinely grown in DMEM supplemented with 10% Fetal Bovine Serum (FBS),

227   2 mM L-alanyl-L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin (Sigma-Aldrich Corp. St.

228   Louis, MO, USA). Cells were incubated with the virus for 45 minutes, washed three times with HBSS^{++}

229   and incubated overnight in DMEM. Infected cells were then fixed in 100% ice cold methanol at 4°C for

230   5 minutes and labeled with influenza A virus nucleoprotein antibody [C43] (Abcam ab128193,

231   Cambridge, MA) 1:200 in HBSS^{++} containing 1% bovine serum albumin (BSA; Sigma-Aldrich Corp.

232   St. Louis, MO, USA) at room temperature for 30 minutes with gentle shaking. Cells were washed three

233   times in HBSS^{++} and labeled with goat anti-mouse Alexa Fluor-488 (Life Technologies, Grand Island,

234   NY) 1:800 in HBSS^{++} containing 1% BSA at room temperature for 30 minutes with gentle shaking.

235   Following three washes in HBSS^{++}, the cells were stained with Vectashield containing DAPI (4',6-

236   diamidino-2-phenylindole) (Victor Laboratories, Burlingame, CA) and observed with the 10x and 40x

237   objectives of an Olympus IX70 fluorescent microscope equipped with a Photometrics PVCAM high-

238   resolution, high-efficiency digital camera. For each sample, at least three fields of view of merged DAPI

239   and Alexa-488 images were acquired. Image-Pro Plus 6.0 software (Media Cybernetics, Bethesda, MD)

240   was used to analyze the 10x images to measure the FFU_{UV} as the ratio of cells infected with the virus

241   divided by the total number of cells.

242   **Data analysis.** The surviving fraction (S) of the virus was calculated by dividing the fraction of cells

243   that yielded positive virus growth at each UV dose (FFU_{UV}) by the fraction at zero dose ($FFU_{controls}$):

244   $S = FFU_{UV}/FFU_{controls}$. Survival values were calculated for each repeat experiment and natural log (ln)

245   transformed to bring the error distribution closer to normal[40]. Linear regression was performed using

246   these normalized ln[S] values as the dependent variable and UV dose (D, mJ/cm^2) as the independent

247   variable. Using this approach, the virus survival (S) was fitted to first-order kinetics according to the

248   equation[7]:

249   $$\ln[S] = -k \times D \,, \tag{1}$$

250   where k is the UV inactivation rate constant or susceptibility factor (cm^2/mJ). The regression was

251   performed with the intercept term set to zero, which represents the definition of 100% relative survival

252   at zero UV dose. Bootstrap 95% confidence intervals for the parameter k were calculated using R 3.2.3

253   software[41]. The virus inactivation cross section, D_{95}, which is the UV dose that inactivates 95% of the

254   exposed virus, was calculated as $D_{95} = -\ln[1 - 0.95]/k$.

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Author Contributions

D.W., M.B., and V.G. designed and performed experiments, analyzed the data, and wrote the manuscript; I.S. analyzed the data; C.C. and A.W.B. designed the irradiation chamber; G.W.J. constructed the irradiation chamber; D.J.B and G.R-P. supervised, contributed conceptual advice, and wrote the manuscript. All authors discussed the results and commented on the manuscript.
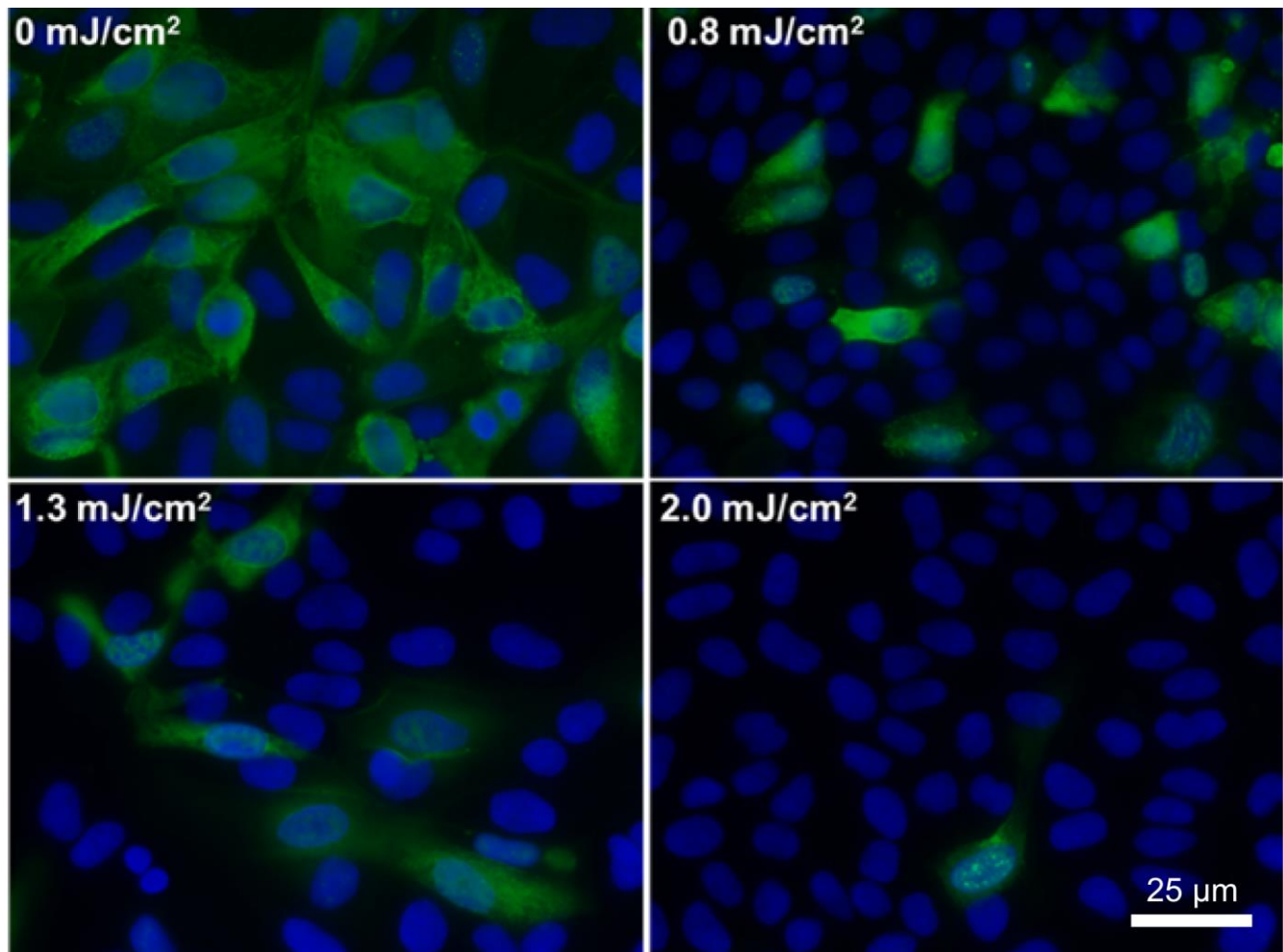
Competing Interests

The authors declare that they have no competing interests.

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363 **Figure 1 Antiviral efficacy of different low doses of 222-nm far-UVC light.** Typical fluorescent

364 images of MDCK epithelial cells infected with influenza A virus (H1N1). The viruses were exposed in

365 aerosolized form in the irradiation chamber to doses of 0, 0.8, 1.3 or 2.0 mJ/cm^2 of 222-nm far-UVC

366 light. Infected cells fluoresce green (blue = nuclear stain DAPI; green= Alexa Fluor-488 conjugated to

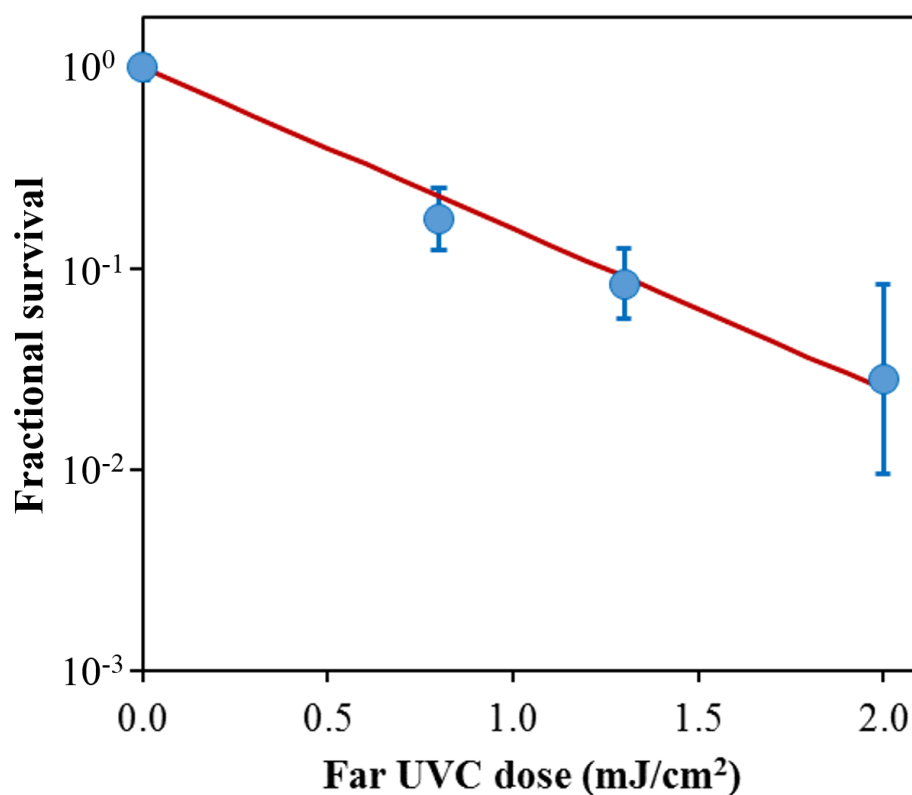367 anti-influenza A antibody). Images were acquired with a 40x objective.

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373 **Figure 2 Quantification of the antiviral efficacy of 222-nm far-UVC light.** Fractional

374 survival, FFU_{UV} / $FFU_{controls}$, is plotted as a function of the 222-nm far-UVC dose. Means and

375 standard deviations refer to triplicate repeat studies and the line represents the best-fit
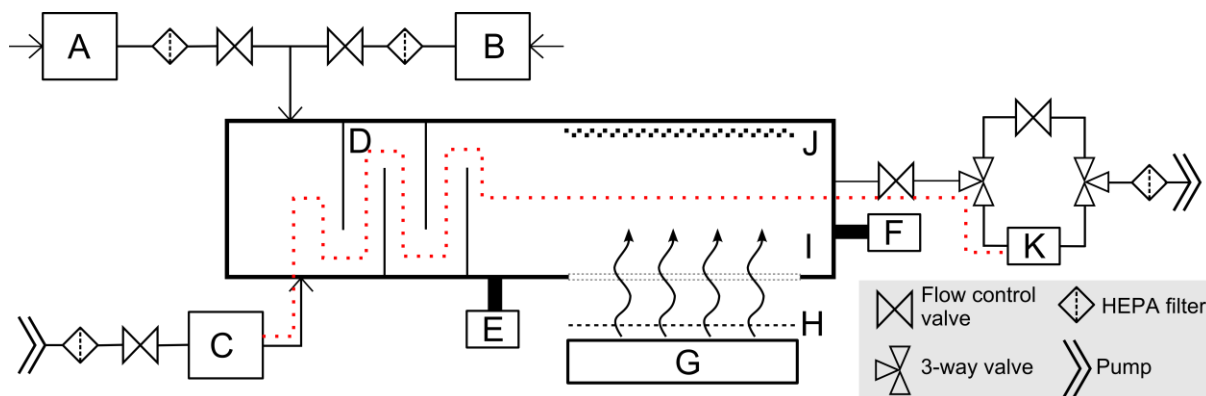
376 regression to Eqn 1 (see text).

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Figure 3 Schematic diagram of the custom UV irradiation chamber. The chamber is depicted in a top down view. Components of the setup include: water bubbler for humidified air input (A), a desiccator for dry air input (B), a nebulizer (C), baffles (D), an RH and temperature meter (E), a particle sizer (F), far-UVC lamps (G), band pass filters (H), a far-UVC transmitting plastic window (I), a reflective aluminum surface (J), and a BioSampler (K). Pumps are used to pressurize the nebulizer for aerosol generation and to control flow through the system. Flow control valves allow adjustments through the system. HEPA filters are included on all air inputs and outputs. A set of three way valves controls flow to or around the BioSampler. The vertically stacked lamps are directed at the window in the side of the chamber to expose the aerosols passing horizontally. The additional films to uniformly decrease the dose were placed between the filters and the window. The path of the aerosolized virus within the system during sampling is indicated with the red dotted line.