1 **Rapid and efficient inactivation of surface dried SARS-CoV-2 by UV-C**

2 **irradiation.**

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29 **Abstract**

30 The SARS-CoV-2 pandemic urges for cheap, reliable and rapid technologies for disinfection

31 and decontamination. We here evaluated the efficiency of UV-C irradiation to inactivate

32 surface dried SARS-CoV-2. Drying for two hours did not have a major impact on the infectivity

33 of SARS-CoV-2, indicating that exhaled virus in droplets or aerosols stays infectious on

34 surfaces at least for a certain amount of time. Strikingly, short exposure of high titer surface

35 dried virus ($3*10^6$ IU/ml) with UV-C light (0.66 mJ/cm²) resulted in a total reduction of SARS-

36 CoV-2 infectivity. Together, our results demonstrate that SARS-CoV-2 is rapidly inactivated

37 by relatively low doses of UV-C irradiation. Hence, UV-C treatment is an effective non-

38 chemical possibility to decontaminate surfaces from high-titer infectious SARS-CoV-2.

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57 **Introduction**

58 SARS-CoV-2 has spread globally and there is an urgent need for rapid, highly efficient,

59 environmentally friendly, and non-chemical disinfection procedures. Application of UV-C light

60 is an established technology for decontamination of surfaces and aerosols (1-3). This

61 procedure has proven effective to inactivate SARS-CoV-1 (4-6), several other enveloped and

62 non-enveloped viruses as well as bacteria (7). Recently, it has also been shown that SARS-

63 CoV-2 is sensitive to inactivation by UV-C irradiation (8-10). However, doses and exposure

64 times necessary for total inactivation of SARS-CoV-2 were in a range precluding efficient

65 application of UV-based methods to be employed for large-scale decontamination of surfaces

66 and aerosols (10). We hence conducted a "real-life" application approach simulating the

67 inactivation of dried surface residing infectious SARS-CoV-2 by a mobile handheld UV-C

68 emitting device and an UV-C box designed to decontaminate medium-size objects. Our data

69 shows that surface dried SARS-CoV-2 retains infectivity for at least two hours. Short

70 exposure of high-titer surface dried SARS-CoV-2 to UV-C light lead to a total reduction of

71 infectivity. Hence, UV-C irradiation is a rapid and cost-effective technology to decontaminate

72 surfaces from high-titer SARS-CoV-2.

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74 **Material and Methods**

75 **Cell culture.** Caco-2 (Human Colorectal adenocarcinoma) cells were cultured at 37 °C with

76 5% CO_2 in DMEM containing 10% FCS, with 2 mM l-glutamine, 100 µg/ml penicillin-

77 streptomycin and 1% NEAA.

78 **Viruses.** The recombinant SCoV2 expressing mNeonGreen (icSARS-CoV-2-mNG) (11) was

79 obtained from the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA)

80 at the UTMB (University of Texas Medical Branch). To generate icSARS-CoV-2-mNG stocks,

81 200,000 Caco-2 cells were infected with 50 µl of virus stock in a 6-well plate, the supernatant

82 was harvested 48 hpi, centrifuged, and stored at -80°C.

83 For MOI determination, a titration using serial dilutions of the virus stock was conducted. The

84 number of infectious virus particles per ml was calculated as the (MOI × cell

85 number)/(infection volume), where MOI = −ln(1 − infection rate).

86 **UV-C light inactivation treatment.** 35 uL of virus stock, corresponding to ~4*10^6 infectious

87 units (IU) of icSARS-CoV-2-mNG were spotted (in triplicates) in 6-well plates and dried for

88 two hours at RT. 6-well plates spotted with dried virus were treated with UV-C-light using the

89 Soluva® pro UV Disinfection Chamber (Heraeus) for 60 seconds or the Soluva® pro UV

90 Disinfection Handheld (Heraeus) for 2 seconds in a fix regime at 5 and 20 cm plate distance.

91 In addition, a moving regime using a slow (3.75 cm/s) and fast (12 cm/s) speed at 20 cm

92 distance was tested. As control, 6-well plates were spotted with the virus and dried, but not

93 UV-treated. After UV-treatment, the spotted virus was reconstituted using 1 mL of infection

94 media (culture media with 5% FCS). As control, 35 uL of the original virus stock were diluted

95 to 1 ml with infection media and used as virus stock infection control.

96 **Evaluation of UV-treatment.** For infection experiments, 1×10^4 Caco-2 cells/well were

97 seeded in 96-well plates the day before infection. Cells were incubated with the SARS-CoV-2

98 strain icSARS-CoV-2-mNG at a MOI=1.1 (stock) or the UV-treated and reconstituted virus in

99 serial two-fold dilutions from 1:200 up to 1:51200. 48 hpi cells were fixed with 2% PFA and

100 stained with Hoechst33342 (1 µg/mL final concentration) for 10 minutes at 37°C. The staining

101 solution was removed and exchanged for PBS. For quantification of infection rates, images

102 were taken with the Cytation3 (Biotek) and Hoechst+ and mNG+ cells were automatically

103 counted by the Gen5 Software (Biotek).

104 Viral titers (number of infectious virus particles per ml) were calculated as the (MOI × cell

105 number)/(infection volume), where MOI = −ln(1 − infection rate). Infection rates lower than

106 0.01 were used as a cutoff and set to 0 in order to avoid false positive calculations.

107 **Software and statistical analysis.** GraphPad Prism 8.0 was used for statistical analyses

108 and to generate graphs. Figures were generated with CorelDrawX7. Other software used

109 included Gen5 v.3.10.

110

111 **Results**

112 We set up an experimental approach to evaluate the effect of UV-C treatment on the stability

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113   of SARS-CoV-2. Simulating the situation that exhaled droplets or aerosols from infected

114   individuals contaminate surfaces, we produced a high-titer SARS-CoV-2 infectious stock and

115   dried 35µL of this stock corresponding to ~$4*10^6$ IU/ml in each well of a 6-well plate. The

116   plates were then either non-treated or exposed to five UV-C regimens (Fig. 1a). These

117   include inactivation for 60 s in a box designed to disinfect medium-size objects, 2 s exposure

118   at 5 cm or 20 cm distance with a handheld UV-C disinfection device and finally an approach

119   simulating decontamination of surfaces via the handheld UV-C device. For this, we performed

120   slow and fast-moving at a distance of ~20 cm, with "slow" corresponding to a speed of ~3.75

121   cm/s (supplemental movie 1) and "fast" at ~12 cm/s (supplemental movie 2). UV-C irradiance

122   (254 nm) in the box with an exposure time of 60 seconds corresponds to an irradiation dose

123   of 800 mJ/cm²; for the handheld (HH) at 5 cm the UV-C dose at two second irradiation time is

124   80 mJ/cm² and at 20 cm is 16 mJ/cm². From the speed of the "slow" and "fast" moving

125   regimens we calculate a UV-C dose of 2.13 mJ/cm² (slow) and 0.66 mJ/cm² (fast).

126   Subsequently, dried virus was reconstituted with 1 mL infection media and used to inoculate

127   naïve Caco-2 cells at serial dilutions to calculate viral titers. Taking advantage of an infectious

128   SARS-CoV-2 strain expressing the chromophore mNeonGreen (11), we quantified infected

129   (mNG+) and total (Hoechst+) cells by single-cell counting with an imaging multiplate reader.

130   Of note, even short UV-C treatment of the dried virus in the context of the moving "fast"

131   regimen completely inactivated SARS-CoV-2, as no infected cells were detected based on

132   fluorescence protein expression (Fig. 1b). Titration of two-fold series dilutions of the UV-

133   treated and non-treated control samples, as well as the freshly thawed strain as reference,

134   revealed that (i) drying for two hours does not have a major impact on the infectivity of SARS-

135   CoV-2 and (ii) all five UV-C treatment regimens effectively inactivate SARS-CoV-2 (Fig. 1c).

136   Calculation of viral titers based on the titration of the reconstituted virus stocks revealed a

137   loss of titer due to drying from ~$4*10^6$ to ~$3*10^6$ IU/ml and effective 6-log titer reduction of

138   SARS-CoV-2 by all employed UV-C treatment regimens (Fig. 1d). Altogether, our data

139   demonstrate that UV-C regimens that expose high-titer SARS-CoV-2 to doses down to 0.66

140   mJ/cm² are sufficient to achieve complete inactivation of the virus.

Discussion

Disinfection of surfaces and aerosols by UV-C irradiation is an established, safe and non-chemical procedure used for the environmental control of pathogens (1-3, 12). UV-C treatment has proven effective against several viruses including SARS-CoV-1 (4-6) and other coronaviruses i.e. Canine coronaviruses (13). Hence, as recently demonstrated by others (8-10) and now confirmed by our study it was expected that SARS-CoV-2 is permissive for inactivation by UV-C treatment. One critical question is the suitability of this technology in a "real-life" setting in which the exposure time of surfaces or aerosols should be kept as short as possible to allow for a realistic application, for example in rooms that need to be used frequently as operating rooms or lecture halls. Furthermore, in such a setting, we assume that the virus is exhaled from an infected person by droplets and aerosols, dries on surfaces and hence represents a threat to non-infected individuals. We simulated such a situation and first evaluated if surface dried SARS-CoV-2 is infectious. Drying for two hours, in agreement with previous work (14), did not result in a significant reduction of viral infectivity indicating smear-infections could indeed play a role in the transmission of SARS-CoV-2 (Fig. 1). On the other hand, our virus-preparations are dried in cell culture pH-buffered medium containing FCS, which might stabilize viral particles. Hence, even though this is not the scope of the current study, it will be interesting to evaluate if longer drying or virus-preparations in PBS affect the environmental stability of SARS-CoV-2. Irrespective of the latter, UV-C-exposure of dried high-titer SARS-CoV-2 preparations containing ~$3*10^6$ IU/ml resulted in a complete reduction of viral infectivity (Fig. 1). In this context, it is noteworthy that we achieved a 6-log virus-titer reduction in a setting simulating surface disinfection with a moving handheld device. With the "fast"-moving protocol (see supplemental video 1) we were exposing surfaces at a distance of 20 cm with a speed of 12.5 cm/s resulting in a calculated UV-C dose of 0.66 mJ/cm² at 254 nm. This is substantially less than the previously reported 1048 mJ/cm² necessary to achieve a 6-log reduction in virus titers when exposing aqueous SARS-CoV-2 to UV-C (10). In another study, using a 222 nm UV-LED source, 3 mJ/cm² lead to a 4-log reduction of infectious SARS-CoV-2 (9) and 20 s deep-ultraviolet treatment at 280 nm

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169   corresponding to a dose of 75 mJ/cm² reduced 3-logs (8).

170   Comparing these values to other pathogens, SARS-CoV-2 seems particularly sensitive

171   towards UV-C light. To achieve a 3-log titer reduction, 75-130 mJ/cm² are necessary for

172   adenovirus, 11-28 mJ/cm² for poliovirus, and bacteria as for instance Bacillus subtilis require

173   18-61 mJ/cm² (7). This is in-line with susceptibility of SARS-CoV towards UV-C in aerosols at

174   2.6 mJ/cm², whereas adenovirus or MS2-bacteriophages were resistant to such a treatment

175   (1).

176   Altogether, we establish the effectiveness of UV-C treatment against SARS-CoV-2 in a

177   setting designed to simulate realistic conditions of decontamination. The easy, rapid,

178   chemical-free, and high efficacy of UV-C treatment to inactivate SARS-CoV-2 demonstrates

179   the applicability of this technology in a broad range of possible settings.

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181   **Author contributions**

182   NR and MS designed the experiments; NR performed the experiments with support from RB;

183   NR, RB and MS analyzed the data; NR and MS drafted the figures and wrote the manuscript;

184   MS developed the manuscript to its final form; MS planned and supervised the study; all

185   authors read, edited, and approved the final manuscript.

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187   **Conflict of interest**

188   The authors declare no conflict of interest

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Figure and Legend

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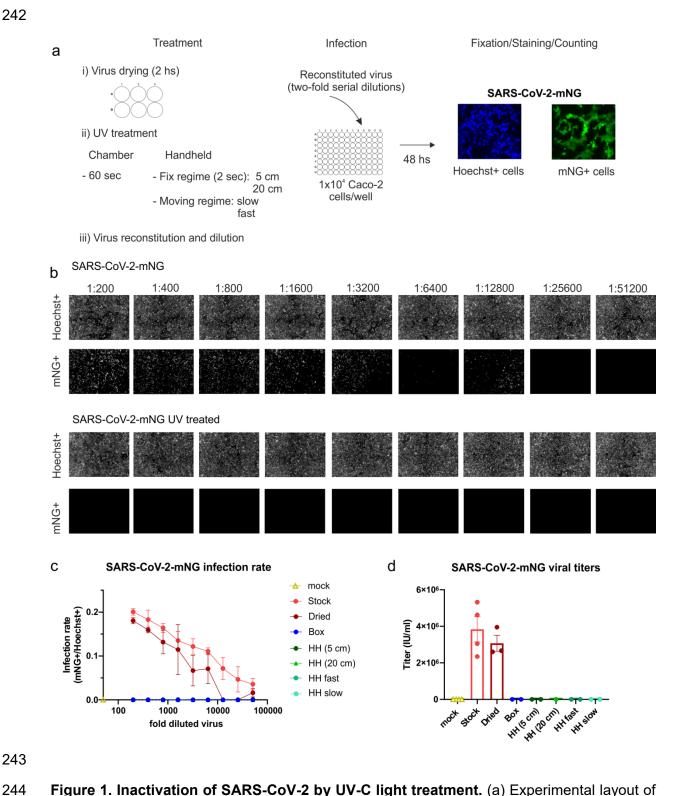

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244 **Figure 1. Inactivation of SARS-CoV-2 by UV-C light treatment.** (a) Experimental layout of

245 the different UV-treatments and the infection assay employed using the green-fluorescent

246 virus SARS-CoV-2.mNG. (b) Primary data showing the results of the infection assay using

247 the non-treated stock virus as a positive control and the UV-treated virus (HH, fast-moving

248 regime). In the upper row, the total amount of cells for each well of the two-fold serial dilution

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249   of virus is shown as Hoechst+. In the lower, infected cells are visualized indicated as mNG+

250   cells. (c) Infection rate curves for UV-irradiated SARS-CoV-2-mNG using different UV-

251   treatments. The graph shows the infection rate at each two-fold serial dilution, calculated as

252   the number of infected cells (mNG+) over the total number of cells (Hoechst+) for the non-

253   treated viral stock (n=4), dried viral stock (n=3), and dried and UV-irradiated virus using five

254   different UV-treatments (n=2). Data are presented as mean +/- SEM of the number of

255   biological replicates indicated above. (d) SARS-CoV-2-mNG viral titers after UV-treatment.

256   The graph shows the viral titers calculated in IU/mL for the mock-infected, non-treated, and

257   dried stock as well as the dried and UV-irradiated virus under the different treatments. The

258   number of biological replicates is directly plotted and indicated in 1c. Data are presented as

259   mean +/- SEM.

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261   **Supplemental Movie 1. UV-irradiation using the Handheld device, slow-moving regime.**

262   SARS-CoV-2-mNG was spotted in a 6-well plate, dried for two hs and UV-irradiated as shown

263   in the video. Speed is calculated at approx. 3.75 cm/s.

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265   **Supplemental Movie 2. UV-irradiation using the Handheld device, fast-moving regime.**

266   SARS-CoV-2-mNG was spotted in a 6-well plate, dried for two hs and UV-irradiated as shown

267   in the video. Speed is calculated at approx. 12.5 cm/s.

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