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1 **Rapid and efficient inactivation of surface dried SARS-CoV-2 by UV-C**
2 **irradiation.**
3

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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.

73

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

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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.

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141 **Discussion**

142 Disinfection of surfaces and aerosols by UV-C irradiation is an established, safe and non-
143 chemical procedure used for the environmental control of pathogens (1-3, 12). UV-C
144 treatment has proven effective against several viruses including SARS-CoV-1 (4-6) and other
145 coronaviruses i.e. Canine coronaviruses (13). Hence, as recently demonstrated by others (8-
146 10) and now confirmed by our study it was expected that SARS-CoV-2 is permissive for
147 inactivation by UV-C treatment. One critical question is the suitability of this technology in a
148 "real-life" setting in which the exposure time of surfaces or aerosols should be kept as short
149 as possible to allow for a realistic application, for example in rooms that need to be used
150 frequently as operating rooms or lecture halls. Furthermore, in such a setting, we assume that
151 the virus is exhaled from an infected person by droplets and aerosols, dries on surfaces and
152 hence represents a threat to non-infected individuals. We simulated such a situation and first
153 evaluated if surface dried SARS-CoV-2 is infectious. Drying for two hours, in agreement with
154 previous work (14), did not result in a significant reduction of viral infectivity indicating smear-
155 infections could indeed play a role in the transmission of SARS-CoV-2 (Fig. 1). On the other
156 hand, our virus-preparations are dried in cell culture pH-buffered medium containing FCS,
157 which might stabilize viral particles. Hence, even though this is not the scope of the current
158 study, it will be interesting to evaluate if longer drying or virus-preparations in PBS affect the
159 environmental stability of SARS-CoV-2. Irrespective of the latter, UV-C-exposure of dried
160 high-titer SARS-CoV-2 preparations containing ~3*10^6 IU/ml resulted in a complete
161 reduction of viral infectivity (Fig. 1). In this context, it is noteworthy that we achieved a 6-log
162 virus-titer reduction in a setting simulating surface disinfection with a moving handheld device.
163 With the "fast"-moving protocol (see supplemental video 1) we were exposing surfaces at a
164 distance of 20 cm with a speed of 12.5 cm/s resulting in a calculated UV-C dose of 0.66
165 mJ/cm² at 254 nm. This is substantially less than the previously reported 1048 mJ/cm²
166 necessary to achieve a 6-log reduction in virus titers when exposing aqueous SARS-CoV-2 to
167 UV-C (10). In another study, using a 222 nm UV-LED source, 3 mJ/cm² lead to a 4-log
168 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/cm^2 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/cm^2 are necessary for
172 adenovirus, 11-28 mJ/cm^2 for poliovirus, and bacteria as for instance Bacillus subtilis require
173 18-61 mJ/cm^2 (7). This is in-line with susceptibility of SARS-CoV towards UV-C in aerosols at
174 2.6 mJ/cm^2, 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.
180

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.
186

187 **Conflict of interest**

188 The authors declare no conflict of interest
189

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196 to publish the data.

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241 **Figure and Legend**

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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.

260

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.

264

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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