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Rapid SARS-CoV-2 disinfection on distant surfaces with UV-C: The inactivation is affected by the type of material

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ABSTRACT

SARS-CoV-2 is responsible for the COVID-19 pandemic, which has caused almost 570 million infections and over six million deaths worldwide. To help curb its spread, solutions using ultraviolet light (UV) for quick virus inactivation inside buildings without human intervention could be very useful to reduce chances of contagion. The UV dose must be sufficient to inactivate the virus considering the different materials in the room, but it should not be too high, not to degrade the environment. In the present study, we have analyzed the ability of a 254 nm wavelength UV-C lamp to inactivate dried samples of SARS-CoV-2 exposed at a distance of two meters, simulating a full-scale scenario. Our results showed that virus inactivation was extremely efficient in most tested materials, which included plastic, metal, wood, and textile, with a UV-C exposure of only 42 s (equivalent to 10 mJ/cm^2). However, porous materials like medium density fibreboard, were hard to decontaminate, indicating that they should be avoided in hospital rooms and public places.

1. Introduction

SARS-CoV-2 is a beta coronavirus responsible for the COVID-19 pandemic, which started in Wuhan (China) in December 2019 and has caused worldwide close to 570 million infections and over six million deaths (data from July 2022, https://www.worldometers.info/coronavirus/). SARS-CoV-2 is a positive strand RNA virus that infects the respiratory tract in humans, causing a range of symptoms that go from a mild cold to severe pneumonia, with a mortality of approximately 1% of infected people, being this percentage notably higher in the elderly [1]. The rapid expansion of this new coronavirus worldwide was due to the fact that it can spread very efficiently from person to person by means of aerosols, especially in closed environments [2]. Several vaccines have been approved for COVID-19, which provide high level protection against infection and severe disease [3]. However, despite vaccination campaigns and the implementation of sanitary measures, such as hand hygiene and the use of face masks, the COVID-19 pandemic is still far from being controlled. Some of the reasons are the low rate of vaccination in some countries, the progressive loss of immune protection conferred by vaccines, the appearance of new SARS-CoV-2 variants that can propagate more efficiently, like B.1.617.2 (delta variant) [4], and especially the B.1.1.529 (omicron variant) [5], and the relaxation of social and hygienical measures.

The most common disinfection measures in public places include the use of chemical agents and exposure to short wavelength ultraviolet radiation (UV-C), which has shown to be very efficient to inactivate SARS-CoV-2 in several laboratory studies [6–12]. Recently, technological solutions combining mobile robotics and UV-C are being developed [13], which could allow virus inactivation without human intervention. This reduces the probability of contagion, and furthermore the movement of the mobile robot could diminish the effect of shadows observed with fixed UV-C lamps. The dose of UV-C to be administered by the robot

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must be sufficient to inactivate the virus, considering the different materials in the room, but it should not be too high to degrade the environment. As the disinfection must be carried out in rooms with different objects and materials, the capacity to inactivate the virus on different materials should be studied.

However, most of these tests have been performed by exposing to UV-C light virus samples deposited on plastic cell culture plates and irradiated at a short distance. These plastic plates do not represent most of the surfaces on which aerosols containing SARS-CoV-2 could deposit contaminating objects of daily use, especially in hospitals where COVID-19 patients are taken care. For that reason, in this study we have analysed the efficacy of UV-C inactivation on SARS-CoV-2 present on the surface of six different materials. We have chosen for this study two types of wood commonly used in furniture, such as medium density fibreboard (MDF) with and without varnish, one type of metal (stainless steel) commonly used in furniture and surgical instruments, two types of plastic, and one type of cloth (white cotton). Plastics include polyvinyl chloride (PVC), commonly used in flooring, plumbing, bottles, etc. and polystyrene, commonly used in packaging, containers, lids, and bottles. Polystyrene is also frequently used to produce laboratory plasticware, such as cell culture plates, being the material most commonly used in previous SARS-CoV-2 inactivation studies [6–9].

2. Material and methods

2.1. Cells and virus

Vero-E6 (ATCC® CRL-1586™) cells, which are susceptible to SARS-CoV-2 infection [14], were grown with Minimum Essential Medium (MEM, Gibco, Waltham, MA) containing 10% foetal bovine serum and antibiotics (MEM complete medium). SARS-CoV-2 virus (isolate NAVARRA-2473) was isolated in April 2020 from the nasal sample of a COVID-19 patient that was hospitalized in the University of Navarra Clinic (Pamplona, Spain) [15], after obtaining the patient's informed consent and the Regional Government permits. The virus was grown in confluent Vero-E6 cells, collecting the supernatant at 72 h post-inoculation, when a clear cytopathic effect was already observed. The virus was filtered, frozen at −80ªC, and one aliquot was thawed and titrated in a plaque assay using confluent Vero-E6 cell monolayers, resulting in a titre of 4.3×10^7 plaque forming units/ml. For virus inactivation experiments we added 10^5 PFUs of SARS-CoV-2 diluted in 0.5 ml of infection medium (MEM with 0.2% bovine serum albumin, 2 mM glutamine and 20 mM Hepes) to sterile polystyrene 100 mm culture dishes (Corning, Corning, NY). The virus concentration used for drying (2×10^5 PFU/ml) was approximately 10-fold higher than that present in droplets from highly-infectious individuals, for whom a maximum of 1.8×10^4 PFU/ml of SARS-CoV-2 was detected in nasopharingeal swabs [16]. We spreaded the virus inoculum on each plate using a spatula and let it dry inside a biosafety hood for 30 min. When drying the virus on other materials (medium density fibreboard with and without varnish, stainless steel, polyvinyl chloride, and white cotton), pieces of these materials of approximately 6 × 6 cm were placed inside 100 mm culture dishes and sterilized with a hydrogen peroxide decontamination-sterilization pass camera before adding the virus to materials inside the hood. All the procedures involving the use of SARS-CoV-2 samples were performed in a BSL3 (P3 Security) laboratory at Cima Universidad de Navarra after obtaining the appropriate authorizations from the Government of Navarra, Spain.

2.2. Plaque forming assay

Determination of the number of SARS-Cov-2 plaque forming units (PFUs) was carried out by infecting confluent Vero-E6 cells in 12-well plates with serial dilutions of virus samples prepared in infection medium. Briefly, cells were washed once with PBS with Ca^{2+} Mg^{2+} (Thermo Fisher, Waltham, MA) and virus dilutions were added to cells. After 1 h of adsorption, medium was removed and overlay medium was added (MEM complete medium and MEM-0.2% agarose (Pronadisa, Torrejón de Ardoz, Spain), [1:1]). Cells were incubated for 72 h at 37 °C, fixed with 0.5% glutaraldehyde (Sigma, St. Louis, MO), and stained with 0.1% crystal violet in methanol-H_2O (20:80). Plates were washed with water and plaques were counted in the appropriate dilution to calculate the PFU/ml titer.

2.3. UV-C lamp and irradiation

For irradiation experiments one of the tubes of the Zenzoe lamp from Asti Mobile Robotics and Boos Technical Lighting, Spain (Spain) was used (TUV 145 W 64T5 HO 4P SE UNP/32 T5). The Zenzoe lamp integrates five fluorescent UV-C tubes, emitting at a 254 nm wavelength with a consumption power of 140 W and a radiant power of 45 W. The lamp glass filters out the 185 nm ozone-forming radiation. The lamp is a device which aims to disinfect surfaces autonomously through UV-C radiation to provide sterile conditions in hospital, industrial, and public environments. One individual lamp from the robot was placed in the BSL3 laboratory at Cima Universidad de Navarra, in a vertical position at a distance of two meters from the biosafety hood, inside which virus samples were exposed on plates placed in a perpendicular position to the light. To calculate the energy reaching the plates we used a LS126C UV light meter (Shenzhen Linshang Technology, Shenzhen, China) which was placed at exactly the same location and distance from the UV lamp than virus samples. The environmental conditions in the laboratory where virus UV inactivation was performed were 20 °C and 68.7% relative humidity.

2.4. Surface microstructure images

Images of the surfaces' microstructure of the different materials that were used were obtained using a Phenom G2 26 PRO SEM microscope.

3. Results and discussion

To study the UV-C inactivation of SARS-CoV-2, we first isolated and titrated this virus from the nasal sample of a COVID-19 patient as described in the Methods section. Our aim was to perform UV-C inactivation studies using samples of SARS-CoV-2 dried on different materials since: (i) this would be the most common situation in real life, where aerosols will quickly dry once deposited on a surface [17] (ii) it allows to place viral samples in a vertical position inside a safety hood so they can be exposed to a UV-C source located outside. Since drying could affect the viability of SARS-CoV-2, we first analysed the recovery of viable virus by drying it on commonly used polystyrene plates. We plated 10^5 PFUs of SARS-CoV-2 on 100 mm plates diluted in a total of 0.5 ml of PBS or MEM 0.2% BSA 2 mM glutamine (MEM-BSA). We let the samples dry completely for 30 min, resuspended the virus in 5 ml of MEM-BSA, and titrated it on Vero-E6 cells using a lysis plate assay. Interestingly, when the virus was dried in PBS, >99% of the infectious virus was lost [mean ± standard deviation (SD) of percentage recovery: 0.11±0.19, $n = 3$]. However, by drying the virus in MEM-BSA we could recover approximately 7% of the initial infectious material (mean ± SD of percentage recovery: 6.8 ± 2.1, $n = 9$). For this reason, this drying strategy was chosen for all subsequent experiments. This recovery is relatively lower than the one described in a previous study [18], although in that case the amount of virus that was dried was about 50-fold higher. A concentration effect on the recovery of virus after drying has been previously reported with other enveloped viruses, such as hepatitis C virus [19]. However, in another report where an amount of SARS-CoV-2 similar to ours was used for drying, recovery from surfaces, such as plastic, steel, and glass, was lower than 1%, although in this case they waited 6 h for the virus to dry [12]. The extremely low recovery observed when drying the virus in PBS could be due to the formation of small crystals due to precipitation of salts. It has been described that the

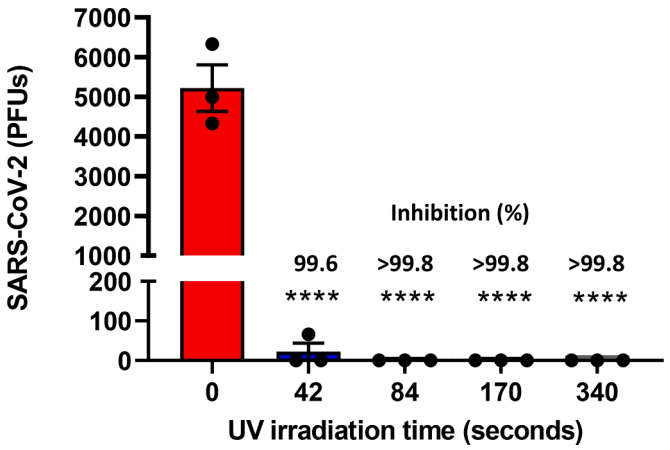

Fig. 1. Evaluation of UV-C inactivation of SARS-CoV-2 with different irradiation times. 10^5 PFUs of SARS-CoV-2 were deposited on polystyrene plates and dried. After drying, viral samples were exposed to a 254 nm UV-C light for the indicated times at a distance of 2 m. Virus was eluted in 5 ml of MEM-BSA and titrated by lysis plate assay on Vero-E6 cells. Data show mean ± SEM. An unpaired t-test was used for comparisons using Prism GraphPad 9 (each condition was compared to unexposed virus). ****, $p<0.0001$.

formation of harmful crystals (either salt or ice) could potentially damage membranes of cells, and enveloped viruses, compromising their integrity [20]. In fact, protocols for freeze-drying of viruses include the use of stabilizers such as disaccharides (i.e. sucrose and trehalose), sorbitol, and animal-derived components, such as gelatin or albumin [21]. The harmful effect of salts was probably attenuated in the presence of the BSA present in the MEM medium that we used for drying the virus.

For inactivation assays we used a 254 nm fluorescent UV-C vertical tube, which is part of an UV-C disinfection autonomous robot. The tube was placed at two meters from the dried virus samples, which were positioned vertically inside a biosafety hood. Before proceeding to analyze the inactivation of SARS-CoV-2 on different materials, we determined the optimal amount of radiation needed to achieve a complete virus inactivation using 10^5 PFUs of SARS-CoV-2 dried on polystyrene plates. Triplicate viral samples were irradiated with 0, 10, 20, 40, and 80 mJ/cm^2 (equivalent to an exposure of 0, 42, 84, 170, and 370 s at two meters). Viral samples were then resuspended in 5 ml of MEM-BSA and titrated as described before. As observed in Fig. 1, 10 mJ/cm^2 was enough to inactivate >99.5% of the infectious virus, being this condition the one chosen to evaluate inactivation of SARS-CoV-2 on different materials. This dose is comparable to that described to inactivate other RNA viruses, like the MS2 bacteriophage, which ranged from 2.51 to 6.50 mJ/cm^2 for 99% viral reduction [22].

We then proceeded to irradiate with 10 mJ/cm^2 10^5 PFUs of SARS-CoV-2 previously dried on the surface of the six different materials mentioned earlier, which were placed inside 100 mm plates (Fig. 2A). After irradiation, viral samples were eluted by resuspending them in 5 ml of MEM-BSA and analyzed to quantify viable virus as described before (Fig. 2B and Table 1). UV-C inactivation of SARS-CoV-2 on both types of plastics, polystyrene and PVC, was very high, reaching >99.5%. These results agree with previous reports in which dried samples of SARS-CoV-2 were very efficiently inactivated on plastic plates [7,13,23,24]. The viruses exposed on stainless steel and varnished MDF were also inactivated very efficiently (93.1% and 90.1%, respectively). However,

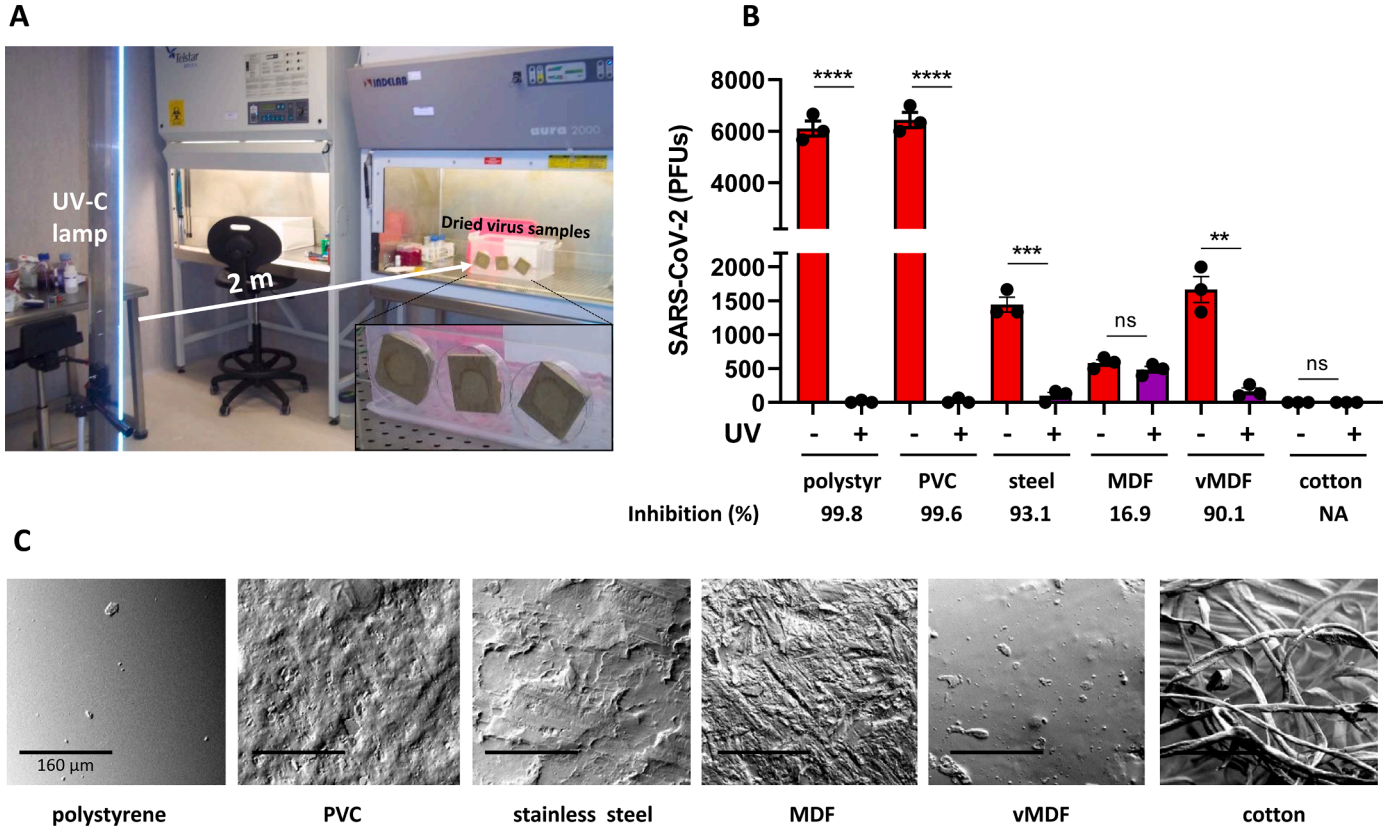

Fig. 2. Evaluation of UV-C inactivation of SARS-CoV-2 on different materials. 10^5 PFUs of SARS-CoV-2 were deposited on the indicated materials and dried. After drying, viral samples were exposed to a 254 nm UV-C light during 42 s at a distance of 2 m as shown in **A** (pieces of medium density fibreboard (MDF) with dried virus are shown in the inset). After UV-C irradiation the virus was eluted in 5 ml of MEM-BSA and titrated by lysis plate assay on Vero-E6 cells (**B**). **C**, Micrographs of the different surfaces used in the assay. polystyr, polystyrene; vMDF, varnished MDF. Data show mean ± SEM. An unpaired t-test was used for comparisons using Prism GraphPad 9 (in B, each condition was compared to unexposed virus). **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$; ns, non-significant.

Table 1
SARS-CoV-2 recovery from different materials.

Material[a]	Recovery (%)	Recovery after UV (%)
Polystyrene	6.25±0.35	0.014±0.01
PVC	6.25±0.23	0.029±0.03
MDF	0.61±0.04	0.513±0.04
vMDF	1.55±0.19	0.132±0.03
Stainless steel	1.36±0.06	0.088±0.08
Cotton	0.00±0.00	0.000±0.00

[a] MDF, Medium density fibreboard; vMDF, varnished MDF.

in the absence of UV-C radiation a lower amount of viable virus could be recovered from these two materials (23.6% and 27.2% of the amount of virus recovered from polystyrene plates for steel and varnished MDF, respectively, Table 1). In studies performed with phage ϕ6, as a surrogate for SARS-CoV-2, virus inactivation on white-melamine faced chipboard sheets (similar to varnished wood) was also similar to that observed for stainless steel [25]. Furthermore, and in agreement with our results, SARS-CoV-2 deposited on plastic and steel surfaces had a titer reduction of approximately 2–3 logs after irradiation with an UV-C dose of 10.25 mJ/cm^2 very similar to ours [12]. In this last study by increasing the UV-C dose to 23.71 mJ/cm^2 a 4-log reduction in titre was reached. Interestingly, no significant inactivation of SARS-CoV-2 was observed in MDF, although the amount of viable virus that was recovered from this material was only 9.5% compared to polystyrene. No infectious virus could be recovered from cotton, probably because the virus gets trapped in this type of material. In agreement with this result, it has been described that infectious SARS-CoV-2 virus could not be recovered from tissue paper and cloth [24] It has also been suggesting that the virus may be inactivated more efficiently when dried on water absorbent porous materials [26]. The lack of SARS-CoV-2 inactivation on MDF could be due to the porous nature of this material. This could result in most viral particles being "hidden" from UV-C light. In fact, when MDF is covered by varnish, thus eliminating the porosity on the surface, the virus was efficiently inactivated (see a microscopic comparison of different surfaces in Fig. 2C). Lack of SARS-CoV-2 inactivation on wood had also been previously reported [27], although the effect of varnishing was not evaluated. We think that these results are important because they demonstrate that porous materials, such as MDF, could be hard to decontaminate by UV-C radiation.

4. Conclusions

This study shows that UV-C light can efficiently inactivate dry samples of SARS-CoV-2 present on different materials. This inactivation took place with a very short exposure time and at considerable distance, something could be achieved by using a mobile robot carrying a UV-C as the one described here. Inactivation on porous materials was not efficient, suggesting that the use of this type of material should be avoided in hospitals and public places.

CRediT authorship contribution statement

Cristina Olagüe: Methodology, Validation, Investigation. **Oihane Mitxelena-Iribarren:** Conceptualization, Methodology, Writing – review & editing. **J.Enrique Sierra-García:** Conceptualization, Resources, Writing – review & editing, Funding acquisition. **Fernando Rodriguez-Merino:** Conceptualization, Writing – review & editing. **Sheila Maestro:** Methodology, Investigation. **Eva Pérez-Lorenzo:** Methodology. **Francisco Guillen-Grima:** Conceptualization, Writing – review & editing. **Gloria González-Aseguinolaza:** Supervision. **Sergio Arana:** Conceptualization, Resources, Writing – review & editing, Funding acquisition. **Cristian Smerdou:** Conceptualization, Validation, Investigation, Resources, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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