Simulated sunlight decreases the viability of SARS-CoV-2

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Research Article

Keywords: COVID-19, SARS-CoV-2, viability, artificial sunlight, sun, solar simulator

DOI: https://doi.org/10.21203/rs.3.rs-37057/v1

Abstract

The novel coronavirus, SARS-CoV-2, has spread into a pandemic since its emergence in Wuhan, China in December of 2019. This has been facilitated by its high transmissibility within the human population and its ability to remain viable on inanimate surfaces for an extended period. To address the latter, we examined the ability of sunlight to degrade SARS-CoV-2 on stainless steel. All assays were performed using a solar simulator at the equivalent of one air mass (i.e. equatorial sun at its Zenith). Heat-controlled experiments were conducted at approximately 34% relative humidity (RH); otherwise, RH decreased with sunlight exposure until a constant temperature was maintained. When initially suspended in tissue culture medium, the virus was rendered non-viable after two hours of sunlight exposure. However, when suspended in an organic matrix designed to mimic bodily secretions, three hours of continuous sunlight was required for complete degradation. From this work, we demonstrate that sunlight represents an effective decontamination method but the speed of decontamination is variable based on the underlying matrix. This information has an important impact on the development of infection prevention and control protocols to reduce the spread of this deadly pathogen.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV–2) is a novel coronavirus which emerged in the city of Wuhan, Hubei province, China in December of 2019[1]. The resultant disease, COVID–19, has since afflicted millions and killed hundreds of thousands throughout the world[2,3,4,5,6]. Efforts to contain the spread of the virus have required whole-of-society mobilization efforts, such as physical distancing and business closures, which have led to worldwide economic devastation. The need for such measures has been driven in part by the ability of this virus to be transmitted by asymptomatic carriers and pre-symptomatic patients[7,8,9]. Furthermore, the role of fomites may also be important, as the virus can remain infectious on some surfaces, such as plastic, glass, and steel, for up to four days[10]. One way to reduce the spread of disease is to regularly disinfect contaminated surfaces with biocidal agents, especially in high traffic areas like public transit stations and emergency rooms. However, this technique is not practical for outdoor surfaces as, generally, there is no one employed to regularly clean them. To address this need, we have examined the ability of sunlight to act as a natural sterilizing medium and reduce the viability of SARS-CoV–2.

Results

Carrier Tests

All of the experiments employed control carriers that were inoculated with virus and maintained within the same biosafety cabinet (BSC). Importantly, all of the carriers demonstrated a relatively stable infectious load over the

course of our experiment, as expected from other studies of environmental stability[10,11,12,13]. Figure 1 demonstrates that the viability of SARS-CoV–2 decreased the longer the virus was exposed to sunlight. Inactivation occurred most efficiently when the virus was suspended in culture medium. Under these conditions, in heat-controlled experiments the virus measured at 1.75×10^3 at time-point (TP) 0 and showed no signs of degradation after one hour on control carriers. However, SARS-CoV–2 was rendered inactive after 60 minutes of sunlight exposure when carrier heat was maintained constant at 22.5°C (relative humidity (RH) ~34%). When carrier temperature was allowed to rise with a concomitant decrease in RH, the viability of sunlight-exposed virus was extended to 120 minutes. Here the titre of viable SARS-CoV–2 was reduced to below the limit of quantification, while the titre of control virus fell only slightly from 9.68×10^3 to 1.39×10^3.

Notably, the presence of an organic matrix extended the survival of the virus when exposed to sunlight. Under these conditions, sunlight exposure for three hours was necessary to fully inactivate the virus across all biological replicates in both heat-controlled and heat-permitted assays. The viability of virus recovered from control carriers did not decrease over the same time span (from 3.98×10^3 at TP0 to 4.88×10^3) when heat was maintained constant and fell only slightly when carrier temperature rose and RH declined (from 3.98×10^3 to 1.37×10^3).

Discussion

The medical, social and economic impacts of COVID–19 have raised important questions regarding how to safely reopen society. One critical question is the risk posed by fomites in outdoor spaces. Other studies have demonstrated that COVID–19 is able to survive on a variety of surfaces and remain infectious[10,11,12,13]. Our study demonstrates that the viability of SARS-CoV–2 can be significantly reduced by exposure to solar radiation. Notably, infectivity of the virus remains relatively constant in the absence of solar rays in both experiments during the same time span. Importantly, however, we demonstrate that there are important additional factors that affect the efficacy of sunlight in reducing infectivity.

First, the matrix within which the virus is suspended has a demonstrable impact on the effect of sunlight as a disinfection agent. When culture medium was used, infectious virus was no longer detected one hour after sunlight exposure. By contrast, suspending SARS-CoV–2 in an organic matrix appeared to be somewhat protective, with detection of very low viral titres after two hours of exposure to sunlight. This is not surprising, as the organic matrix comprises three types of protein (high molecular weight proteins, low molecular weight peptides, and mucous material), designed to represent bodily secretions[14] which may insulate the virus. By three hours, however, SARS-CoV–2 is no longer viable in the matrix. Given that most shed virus is excreted within mucus, the longer exposure time appears more relevant to implementation of these findings.

A second important finding is that both heat and humidity impacts virus survival. When carrier heat was kept constant (22.5°C; RH ~34%), viral viability decreased to below the limit of quantitation (LOQ) after one hour of sunlight exposure. Interestingly, when the steel carriers were allowed to heat up from the light exposure and a concomitant decrease in RH ensued, the period of viability was extended, requiring two hours of exposure to achieve the same result. The same effects were observed for the control virus, as a decrease in viability, albeit slight, was only observed in heat-controlled experiments where higher RH was higher (~34%). The validity of these findings is supported by observations that higher levels of humidity lead to lower incidences of COVID–19 transmission and mortality[15,16,17]. Since the heat and humidity conditions did impact survival, it is important to consider environmental conditions when determining decontamination protocols. We would favour the latter scenario (i.e. variable heat and variable humidity) as more representative of real-world conditions.

Our findings are important in demonstrating that sunlight can be used to decontaminate surfaces confirmed or suspected of having been exposed to SARS-CoV–2. However, our study has important limitations. First, we examined sunlight conditions equivalent to a sun at equatorial latitudes in the absence of cloud cover. As solar intensity varies geographically, it would be important to adjust exposure times to deliver a similar solar radiation dose based on local conditions. A second limitation is the use of a non-porous surface for these experiments. It is known that surface characteristics can also impact survival of the virus, with non-permeable surfaces allowing the virus to persist longer than do absorbent materials[10,11,12,13]. Finally, we examined simulated mucus but no other spiked or simulated bodily fluids. SARS-CoV–2 RNA has been detected routinely from patients, but recovering infectious virus appears to

be much less frequent[18,19]. In spite of this, it would be worthwhile to examine the effect of sunlight on SARS-CoV–2 in other matrices (e.g. naso-/oro-pharyngeal fluids, stool, etc.) where infectious virus has been recovered[18,19].

Overall, these findings are important in determining plans for the maintenance and decontamination of outdoor spaces as public health measures are relaxed. Sunlight does appear effective in reducing levels of infectious virus following three hours of exposure when embedded within mucus. Removal of mucus through surface cleaning would be expected to increase the efficiency of viral decontamination. Careful attention to total solar dose and RH should be considered, since these factors affect the rate of decontamination. Further work to explore other surfaces and environmental conditions should be performed.

Methods

Virus Propagation

The initial virus aliquot of SARS-CoV-2 (cultured from patient sample; viral passage 1; *hCoV-19/Canada/ON-VIDO-01/2020, GISAID accession# EPI_ISL_425177)* was provided by the Vaccine and Infectious Disease Organization (VIDO; Saskatoon, Saskatchewan, Canada). Vero E6 cells were grown in 150 cm^2 tissue-cultured treated flasks to 80-90% confluence in Dulbeco's Minimum Essential Medium (DMEM) supplemented with 5% bovine calf serum (BCS). Within a biosafety level (BSL)-3 laboratory, the medium was removed and the cells washed with DMEM containing 0.1% bovine serum albumin (BSA). The cells were then infected with the SARS-CoV-2 aliquot (5 µl) in DMEM (5 ml) containing 0.5 µg/ml TPCK-Trypsin and 0.1% BSA and incubated at 37°C and 5% CO_2. After 30 minutes of absorption with intermittent rocking every 5-10 minutes, additional maintenance medium (30 ml) was added and the cells were again incubated at 37°C and 5% CO_2. Any resulting cytopathic effect (CPE) was monitored daily, with the supernatant harvested five days post-infection (dpi). The initial virus inoculum was quantified by end-point titration on Vero E6 cells and determined to be $4.6x10^6$ $TCID_{50}$/ml (i.e. 50% tissue culture infectious dose per milliliter).

Organic Matrix

The organic matrix used in this study is the standard tripartite soil load described in ASTM E2197-17 e1[14]. Exceptionally, the mucin suspension in 0.85% NaCl was gamma-irradiated at 2 MRads on wet ice as an alternative to filter-sterilization to avoid clogging of the filter.

Solar Simulator

The artificial sunlight used in this study was produced by the SunLite Solar Simulator Model 11002 from Abet Technologies. Solar output was set to 1 sun, equivalent to 1 air mass or natural sunlight emitted at the equator during peak hours on a cloudless day. An atmospheric edge filter was used to block all wavelengths below 305 nm, as radiation below this level is absorbed by the atmosphere in the natural environment.

Carrier Tests

Vero cells were seeded into clear, flat-bottomed, tissue-culture treated 96-well plates in Minimum Essential Medium (MEM; 100 µl) supplemented with 5% BCS and 1% L-glutamine, and grown to approximately 90% confluence overnight at 37°C and 5% CO_2. All subsequent procedures were performed in a BSL-4 laboratory in a class II biological safety cabinet by workers wearing positive-pressure ILC Dover suits. One stock vial of SARS-CoV-2 was thawed at room temperature and 340 µl added to 160 µl of either maintenance medium (MEM containing 1% BCS and 1% L-glutamine) which emulates the virus in a laboratory environment, or organic matrix which emulates the virus in its natural environment[20]. Positive controls were prepared in triplicate by adding the viral suspension (10 µl) to maintenance medium (1 ml). Carriers were prepared by adding the viral suspension (10 µl) to the centre of sterilized stainless steel disks (1 cm in diameter and 0.7 mm thick) and allowed to dry for 45 minutes. Carriers were prepared in triplicate for test and control conditions at each designated time point. Once dry (i.e. TP0), maintenance medium (1 ml) was pipetted up and down on each of three carriers to re-suspend the virus, and used to infect Vero cells (see following paragraph). In experiments designed to control for the confounding variable of heat, half of the carriers were placed directly under the solar simulator light source set to 1 sun in a digital block heater/cooler set to 14°C, which ensured the carriers remained at room temperature (22.5°C). Corresponding carriers were placed in a petri dish within the BSC. For experiments where infrared heat was permitted, control carriers were placed in the block heater/cooler and heated to the same temperature the disks reached under the solar simulator, which was periodically measured with a thermometer and wire probe.

Shortly after collection, each sample and positive control were used to infect the nearly confluent Vero cells in triplicate: after removal of the growth medium, neat virus (100 µl) was added to the top row of the 96-well plate and a series of virus dilutions (10^{-1} to 10^{-6}) prepared in maintenance medium were added to the six consecutive rows below. The wells in the bottom row of the plate contained maintenance medium only and served as negative controls. The plates were incubated at 37°C and 5% CO_2 for four days, at which time individual wells were examined for CPE. The Reed and Muench calculation[21] was used to calculate $TCID_{50}$/ml values and the LOQ. Log10 $TCID_{50}$/ml values were then calculated and plotted to examine virus viability in all treatment groups over time.

Declarations

Author Contributions

A.S. conducted and analyzed carrier tests, and wrote the manuscript; T.C. conducted and analyzed carrier tests; B.D.G. analyzed carrier tests; S.K. conducted carrier tests; Z.S. and M.C. performed cell culture and reagent preparation; J.A. aided in analysis of results; A.L. and D.K. propagated the SARS-CoV-2 virus; D.R.S. provided invaluable expertise and guidance; G.P. provided oversight into all functions of this study and edited the manuscript. All authors reviewed the manuscript.

Additional Information

The authors declare that no competing interests exist.

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Figures

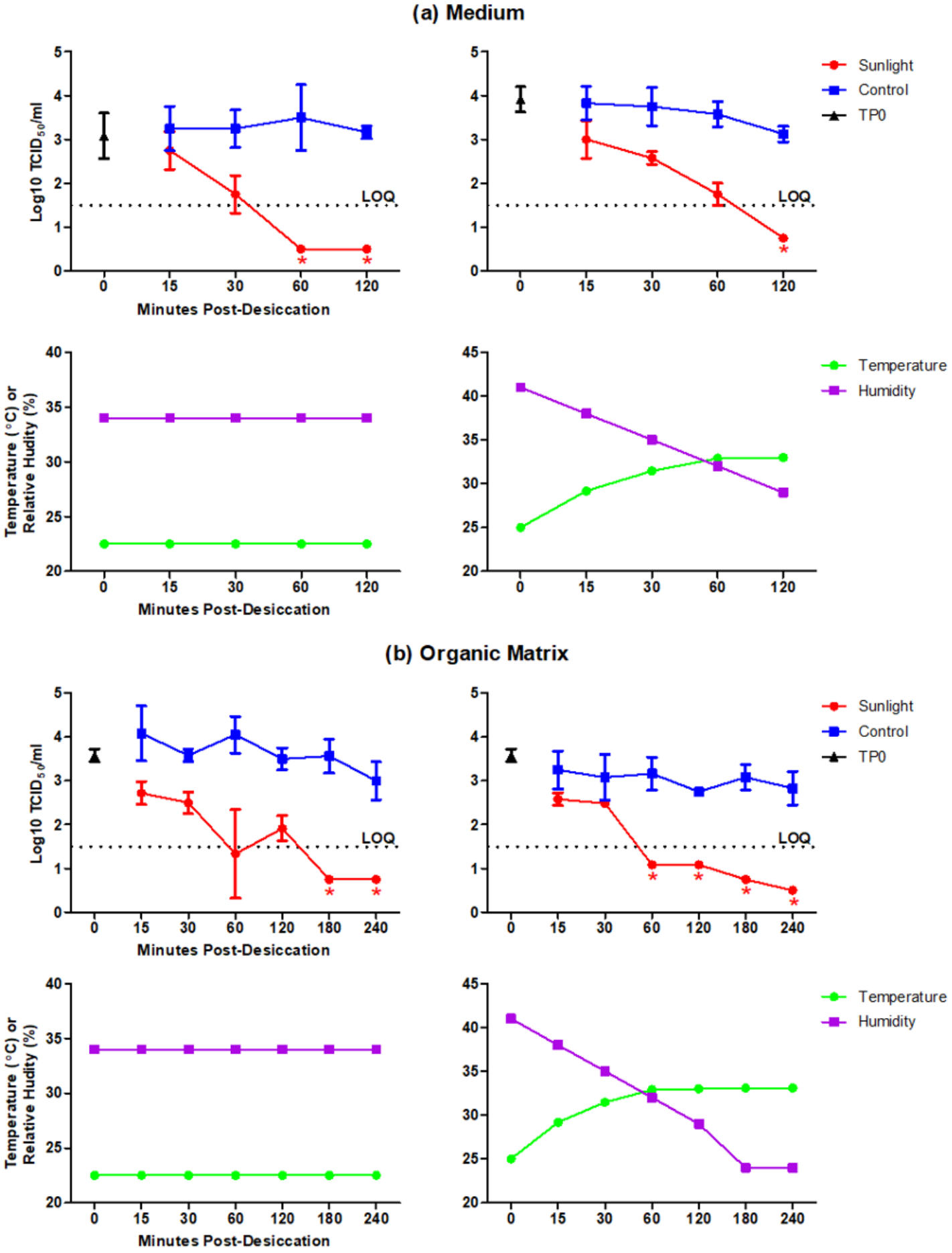
(a) Medium
Log10 TCID50/ml
LOQ
Minutes Post-Desiccation
Sunlight
Control
TP0
Temperature (°C) or Relative Hudity (%)
Temperature
Humidity
(b) Organic Matrix

Figure 1

Viability of SARS-CoV-2 on stainless steel after exposure to simulated sunlight. SARS-CoV-2 was suspended in (a) culture medium or (b) an organic matrix, deposited on stainless steel, desiccated (“TP0”), and exposed to either simulated sunlight (“Sunlight”) or corresponding ambient conditions (“Control”). Graphs in the top row of (a) and (b) show the titer of viable eluted virus, expressed as the Log10 50% tissue culture infectious dose per milliliter (TCID50/ml), following culture in Vero cells. The limit of quantitation (LOQ), denoted by a dashed line, is 1.5 logs or 3.16 x 101 TCID50/ml. Plots show the mean and standard deviation of three biological replicates per time-point, with each biological replicate representing the average of three technical replicates. Plot points denoted by an asterisk were not quantifiable and assigned values for graphing purposes only; consequently, standard deviation could not be calculated for these data. Graphs in the bottom row of (a) and (b) show the carrier temperature and relative humidity readings measured at each time-point, depicting heat-controlled (left) and heat-permitted (right) assays, and correspond to the experiment represented in the graph located directly above.