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Major Article

Treatment with dry hydrogen peroxide accelerates the decay of severe acute syndrome coronavirus-2 on non-porous hard surfaces

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Background: Disinfection of contaminated or potentially contaminated surfaces has become an integral part of the mitigation strategies for controlling coronavirus disease 2019. Whilst a broad range of disinfectants are effective in inactivating severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), application of disinfectants has a low throughput in areas that receive treatments. Disinfection of large surface areas often involves the use of reactive microbiocidal materials, including ultraviolet germicidal irradiation, chlorine dioxide, and hydrogen peroxide vapor. Albeit these methods are highly effective in inactivating SARS-CoV-2, the deployment of these approaches creates unacceptable health hazards and precludes the treatment of occupied indoor spaces using existing disinfection technologies. In this study, the feasibility of using dry hydrogen peroxide (DHP) in inactivating SARS-CoV-2 on contaminated surfaces in large indoor spaces was evaluated.

Methods: Glass slides were inoculated with SARS-CoV-2 and treated with DHP between 5 and 25 ppb for up to 24 hours. Residual infectious virus samples were eluted from three replicates at each time point and titrated in African green monkey VeroE6 cells.

Results: In comparison with the observed relatively high stability of SARS-CoV-2 on contaminated glass slides (control group), residual infectious titers of glass slides inoculated with SARS-CoV-2 were significantly reduced after receiving 120 minutes of DHP treatment.

Conclusions: The accelerated decay of SARS-CoV-2 on contaminated glass slides suggests that treatment with DHP can be an effective surface disinfection method for occupied indoor spaces

The ongoing pandemic of coronavirus disease 2019 (COVID-19) has led to unprecedented disease burden and economic losses worldwide. In addition to the exposure to respiratory droplets from infected individuals, contaminated environments have also been suggested to play a role in the spread of SARS-CoV-2.[1,2] Whilst direct evidence supporting the transmission through contact with a contaminated environment may be hard to establish, this perception is further strengthened by the high environmental stability survival of SARS-CoV-2 and 2 related coronaviruses, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus.[3,4] Therefore, routine cleaning and disinfection of surfaces to inactivate residual viruses has been recommended for households and public spaces.

A range of chemical disinfectants can be used to inactivate SARS-CoV-2 on contaminated surfaces.[5] Manual application of disinfectants has a limited range in the area of surface disinfection. Treatment of large complex surfaces often requires specialized equipment that is operated by highly trained individuals and often involves hazardous materials. Ultraviolet germicidal irradiation (UVGI) is the most commonly used method for environmental disinfection. UV-C at wavelengths between 100 nm and 280 nm have been proven to be

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effective against SARS-CoV-2.[6] However, factors such as distances from the sources of UV-C, dust, and environmental pollutants can limit the germicidal potency.[7] Several chemical disinfectants such as hydrogen peroxide and chlorine dioxide can be vaporized and used to disinfect indoor spaces.[8] Existing environmental disinfection methods often create health hazards during the pathogen inactivation process. With the exception of retrofitting UV-C modules in air filtration systems,[9] the deployment of environmental disinfection equipment to inactivate SARS-CoV-2 in occupied indoor spaces remains impractical.

In this study, we demonstrated the feasibility of inactivating SARS-CoV-2 on dried contaminated surfaces present in large indoor spaces using dry hydrogen peroxide (DHP). In contrast to the health hazards created by the fumigation of indoor spaces with hydrogen peroxide, the release of DHP has been proven to be safe for humans and previously evaluated for the inactivation of bacterial pathogens in healthcare facilities.[10,11] Reduced infectious titers of SARS-CoV-2 recovered from contaminated surfaces after the release of DHP in a 50 m^2 laboratory suggest the potential use of DHP for the environmental disinfection during the ongoing COVID pandemic may be useful.

MATERIALS AND METHODS

Cells, viruses, and quantification of infectious titers

Propagation and quantification of virus stocks and samples were performed using VeroE6 cells.[12] All cultures were maintained in high-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 10% tryptose phosphate broth, penicillin (100 unit/mL), streptomycin (100 μg/mL), and L-glutamine (2 mM). SARS-CoV-2 USA-WA-1/2020 was originally acquired from BEI Resources (Lot number 70033175) and passaged twice to generate the stocks used in this study. Infectious titers of all samples were quantified using median tissue culture infectious dose ($TCID_{50}$) method as previously described.[13]

Generation of contaminated surface and treatment with dry hydrogen peroxide

Dried contaminated surfaces were created using 25 mm (W) x 75 mm (L) x 1 mm (D) microscope glass slides spiked with SARS-CoV-2. Standard glass microscope slides were selected for testing based on the published report on the recovery of infectious viruses up to 48 hours after the inoculation of SARS-CoV-2 and desiccation.[14] Contaminated glass slides were used to determine the decay of residual infectious titers after the initiation of DHP treatment and compared to slides in the control group, which did not receive DHP treatment.

Each glass slide was spiked with 250 μl of SARS-CoV-2 stocks at approximately 6 $\log_{10}TCID_{50}$/mL followed by drying inside a class II A2 biosafety cabinet for one hour. Three contaminated glass slides were randomly assigned as a set, placed inside a 100 mm x 100 mm square petri dishes, sealed with parafilm and transported to two biosafety level-3 large animal (BSL-3Ag) laboratories that are each 50 m^2 in size. The first laboratory was charged with DHP by allowing the continuous operation of a Sentry DHP generator at full output of 38 cubic feet per minute (Synexis LLC, Lenexa, KS) for 24 hours prior to the experiment. This setting generates DHP at a concentration no greater than 25 parts per billion (ppb).[15] The second laboratory was used as a control environment which maintained the negative air pressure required for BSL-3 laboratories. To simulate the air exchange conditions in public spaces, the rate of air exchange in both BSL-3Ag laboratories was reduced to seven air exchanges per hour, the minimal frequency required for the maintenance of negative air pressure. Ambient temperature was maintained at 22°C throughout the experiment. Relative humidity was set at 55% for the entire time course. A total of nine sets of triplicate samples were used in each group to quantify residual infectious viruses present on contaminated glass slides incubated in each room at 0, 15, 30, 45, 60, 90, 120, 240, and 1440 minutes after the exposure to DHP and ambient air. The time course was designed to reflect the stability of SARS-CoV-2 on a variety of contaminated surfaces as residual infectious titers can maintain above the limit of detection of our assay at 1.06 $\log_{10}TCID_{50}$/mL for up to 24 hours.[16] At each designated timepoint, the lid of each petri dish was returned, sealed with clear adhesive tape and kept on ice until the elution of residual viruses.

Elution of residual infectious viruses from contaminated glass slides

Residual infectious viruses were eluted by gently wiping the surface of each contaminated glass slide with a sterile polyester applicator (Fisher Scientific) saturated with Dulbecco's phosphate buffer saline for 20 times. The tip of each applicator was submerged in 1 mL of DMEM media followed by vortexing at 3,000 rpm for 15 seconds to dislodge infectious viruses. All DMEM media containing infectious viruses were filtered using 0.22 μm disc filters to eliminate environmental contaminants and titrated using $TCID_{50}$ method.

Statistical analysis

Residual infectious titers of individual samples collected at designated timepoints were calculated using the Reed-Munich method. Whilst the triplicate samples are a standard practice for environmental stability, the numbers of replicates do not warrant the analysis using repeated measures t-test.[17] Therefore, the kinetics in the decay of infectious titers between two groups was compared using mixed effects model.

RESULTS

Glass slides spiked with SARS-CoV-2 stocks in this study yielded detectable infectious titers after the drying process. The geometric mean titer of infectious viruses eluted at the beginning of this study of 3.6 $\log_{10}TCID_{50}$/mL is comparable to residual infectious titers reported among non-porous surfaces contaminated with SARS-CoV-2. Quantities of residual infectious virus eluted from contaminated glass slides were used to determine the kinetics in the decay of SARS-CoV-2. In the control, non-DHP treated slides, the titers of SARS CoV-2 detected at 24 hour postapplication were not significantly different to the titers of time zero samples. This demonstrates that the virus was environmentally stable under the test conditions and retained infectivity for at least the 24-hour test period. The ability of DHP in inactivating SARS-CoV-2 is demonstrated by the comparison of infectious titers recorded in the control group, which was designed to measure the natural decay of SARS-CoV-2. The comparison of residual infectious titers is summarized in Figure 1.

Infectious titers of samples collected from both the DHP treatment and control groups during the first ninety minutes of this study were indistinguishable from the baseline sample, indicating that SARS-CoV-2 can remain viable on surfaces in the presence of DHP for at least 90 minutes. Exposure to DHP led to the significant reduction of infectious titer, which was observed as early as 120 minutes post the initiation of treatment. Elutes derived from the control group yielded 3.1 $\log_{10}TCID_{50}$/mL of infectious viruses; whereas, glass slides exposure to DHP allowed the recovery of infectious viruses at 1.8 $\log_{10}TCID_{50}$/mL (P= .01). This was consistent with the trend of accelerated decay of SARS-

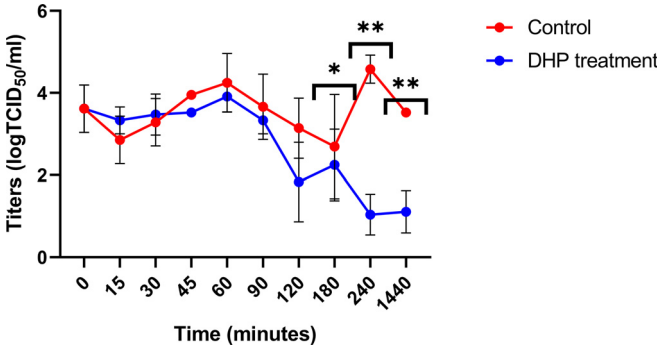

Fig. 1. Dry hydrogen peroxide (DHP) treatment caused the accelerated decay of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Residual infectious titers from glass slides inoculated with SARS-CoV-2 were determined. Geometric mean and standard deviation of infectious units among DHP treatment (blue) and control (red) samples collected at 15, 30, 45, 60, 90, 120, 240, and 1,440 minutes are shown in $\log_{10}TCID_{50}$/mL. Significant reduction of infectious titers was observed in DHP-treated samples obtained at 120, 240, and 1,440 minutes after the initiation of treatment. * and ** indicates *P*-value = .01 and < .0001 based on the results of mixed effects model, respectively.

CoV-2 observed at 240 minutes post the initiation of treatment (control group: 4.6 $\log_{10}TCID_{50}$/mL vs DHP treatment: 1.0 $\log_{10}TCID_{50}$/mL, $P< .0001$).

It is known that residual titers of SARS-CoV-2 on contaminated surfaces incubated at ambient atmosphere can be detected up to 28 days after inoculation.[17] In this study, the continuous DHP treatment for 24 hours led to the inactivation of SARS-CoV-2. Quantities of infectious viruses recovered from glass slides exposed to DHP (1.1 $\log_{10}TCID_{50}$/mL) were significantly lower than those without treatment (3.5 $\log_{10}TCID_{50}$/mL) at this given timepoint ($P< .0001$). It is also noteworthy that 33.3% (1/3) of DHP treated samples did not contain detectable viruses at both 240 and 1440 minutes after the initiation of treatment.

Collectively, SARS-CoV-2 present on contaminated glass slides is sensitive to the treatment of DHP deployed at a sufficiently high concentration. In our study, DHP at 5-25 ppb requires a contact time of 120 minutes to inactivate approximately 98.7% of residual infectious viruses from the initial inoculum containing approximately 3.6 $\log_{10}TCID_{50}$/mL of infectious viruses. In conclusion, our work demonstrates the feasibility of using DHP in reducing the risk of SARS-CoV-2 transmission in occupied indoor spaces.

DISCUSSION

SARS-CoV-2 has an environmental stability that is similar to other betacoronaviruses and gives rise to half-lives of approximately six days on glass and other non-porous surfaces.[17] DHP treatment evaluated in this study led to the accelerated decay of SARS-CoV-2 on contaminated glass slides. Continuous DHP treatment for 120 minutes was required for demonstrable reduction of residual infectious titers. Complete inactivation of SARS-CoV-2 up to 3.6 $\log_{10}TCID_{50}$/mL is likely to require treatments for 240 minutes or longer as observed in this study. The results showed that DHP provides an effective solution for the need of decontaminating occupied indoor spaces during the COVID-19 pandemic. Because the stability of SARS-CoV-2 on contaminated glass is indistinguishable with other non-porous surfaces tested in published studies,[16,17] our results provide the basis for evaluating the feasibility of using DHP to inactivate residual infectious viruses on other materials, including plastic, stainless steel, and vinyl.

Our data are consistent with the previous reports that coronaviruses are relatively environmentally stable but are sensitive to vaporized hydrogen peroxide.[8] Dry fogging of hydrogen peroxide in the form of mixtures containing peroxyacetic acid and hydrogen peroxide has also been demonstrated to be effective in inactivating SARS-CoV-2.[18] In this study, the use of a commercially available portable device can provide a standardized and simplified decontamination procedure for contaminated surfaces containing SARS-CoV-2. The inactivation was achieved using a lower concentration at 5-25 ppb, which is approximately 40 times lower than the concentration of hydrogen peroxide produced using commercially available generators.[8] Although the lower concentration requires a longer contact time, it enables continuous decontamination of occupied indoor spaces. This approach provides an alternative for facilities that are incompatible with the retrofitting of air handling units with UVGI systems in order to decontaminate large surface areas. DHP treatment is also comparable with copper coating technologies developed to inactivate SARS-CoV-2 on non-porous surfaces.[19,20] The deployment of DHP in surface decontamination provides an advantage over copper coating technologies, which require specific instrument or solvent used to promote attachment of copper or copper oxide to different surfaces.

Our study provides the first evidence for the virucidal activity of DHP, which has mainly been validated for the inactivation of human bacterial pathogens in the past.[15] The outcome further justifies the evaluation of DHP in inactivating other enveloped RNA viruses that are transmitted through contaminated surfaces.

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