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Protocols

Rapid thermal inactivation of aerosolized SARS-CoV-2

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ABSTRACT

Airborne transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is one of the leading mechanisms of spread, especially in confined environments. The study aims to assess the thermal inactivation of SARS-CoV-2 at high temperatures in the time scale of seconds. An electric heater with a coiled resistance wire is located perpendicularly to the airflow direction inside an air tunnel. The airflow rate through the tunnel was 0.6 m^3/h (10 L/ min). SARS-CoV-2 were suspended in Dulbecco's modified Eagle's medium (DMEM) with 10 % fetal bovine serum (FBS), aerosolized by a nebulizer at a rate of 0.2 L/min and introduced to the airflow inside the heater with the use of a compressor and an aspirator. In the control experiment, with the heater off, SARS-CoV-2 passed through the system. In the virus inactivation test experiments, the heater's outlet air temperature was set to 150 ± 5 °C and 220 ± 5 °C, and the air traveling through the tunnel was exposed to heat for 1.44 s. An inline gelatine filter harvested SARS-CoV-2 that passed through the system. The viral titer obtained from the gelatine filter in the control experiment was about 5.5 $\log_{10}$ $TCID_{50}$. The virus's loss in viability in test experiments at 150 °C and 220 °C were 99.900 % and 99.999 %, respectively. The results indicate that high-temperature thermal inactivation substantially reduces the concentration of SARS-CoV-2 in the air within seconds.

1. Introduction

The new variants of SARS-CoV-2 emerged in late 2020. A variant of Concern (VOC) 202012/01 has a 43%–90% higher reproduction number than the existing variants (Davies et al., 2021). Possible transmission routes include airborne particles, respiratory droplets, and contact with a contaminated surface. Interrupting the chain of the transmission routes is vital to limit the spread of the virus. One of the inactivation methods is high-temperature exposure, in the range of 56 °C–120 °C, of contaminated surfaces or liquids, reported previously (Darnell et al., 2004; Hessling et al., 2020; Liu, 2004). Heat inactivation of SARS-CoV-2 has mainly been used to sterilize contaminated personal protective equipment such as masks and gloves in hospitals and contaminated equipment and liquids in laboratories before reuse (Abraham et al., 2020; Batéjat et al., 2021; Biryukov et al., 2021).

SARS-CoV-1 in Dulbecco's modified Eagle's medium (DMEM) with supplements was inactivated in 45 min and 75 min at a temperature of 75 °C and 56 °C, respectively (Darnell et al., 2004). A 4 $\log_{10}$ TCID50 reduction of SARS-CoV-2 was observed with a heat treatment protocol of 60 °C for 60 min and 92 °C for 15 min (Pastorino et al., 2020). Using the SARS-CoV and SARS-CoV-2 data from different studies, the time required for 5 $\log_{10}$-reduction was estimated as 32.5, 3.7, and 0.5 min at temperatures of 60 °C and 80 °C, and 100 °C (Hessling et al., 2020). A 6 log10 TCID50 reduction was obtained by increasing the temperature of DMEM to 83.4 °C for 1.03 s using a fluidic system (Jiang et al., 2021). Nevertheless, dry heat inactivation of aerosolized SARS-CoV-2 has not been investigated yet.

During the COVID-19 pandemic, exposure to indoor aerosolized SARS-CoV-2 has become one of the primary challenges (Chia et al., 2020; Morawska et al., 2020). Heat inactivation of the aerosolized SARS-CoV-2 is one way to reduce the spread of 2019 coronavirus disease (COVID-19). In the present study, the inactivation of SARS-CoV-2 at high air temperatures of 150 °C and 220 °C has been investigated using an experimental setup while minimizing potential hazards.

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2. Materials and methods

2.1. Preparation of SARS-CoV-2 suspensions

Experiments were performed in biosafety level 3 (BSL3) facilities of Antimikrop Research and Biocidal Analysis Laboratories, accredited by the Ministry of Health of Turkey. In this laboratory, biocidal analysis tests are routinely performed using SARS-CoV-2. BSL3 virology laboratory is fully equipped with negative pressure vacuum systems, air-lock systems, HEPA filters and biosafety cabinets with HEPA filters (http://www.antimikrop.com.tr/ana-sayfa). A stock suspension of SARS-CoV-2 strain (Gen Bank No: MT955161.1) was used. SARS-CoV-2 virus stock was prepared by inoculating the Vero E6 cell line in Dulbecco's modified Eagle's medium (DMEM-10). Dulbecco's modified Eagle's medium containing supplements (10 % fetal bovine serum, 2 nM/mL L-glutamine, 100 U/mL penicillin, 100 mg/mL streptomycin, and 0.5 mg/mL fungizone (Amphotericin B)) was added to the flask, and the cells were incubated at 37 °C for 72 h. The supernatant was collected, clarified by centrifugation, and stored at −80 °C. $TCID_{50}$ titer was determined by the Spearman- Kärber method as described (Hubert, 1984).

2.2. Experimental setup for heat inactivation of SARS-CoV-2

2.2.1. Experimental setup

The experimental system is shown in Fig.1. An air compressor provided air flow at a rate of 0.6 m^3/h (10 L/min). An air flow meter (RST Measurement Control Tech., Istanbul, Turkey) was used to measure the air flow rate. An electric heater (600 W) was used to heat the air passing through the system. A nebulizer (M102, Jiangsu Yuyue Medical Equipment & Supply Co., Ltd., Danyang Jiangsu, China) aerosolized the virus at a nebulization rate of 0.2 mL/min, and an average particle size of 3.7 microns (as specified by the manufacturer). A venturi injector was used to mix the aerosolized virus to the air flow in the system. An inline polycarbonate filter holder with a gelatin membrane filter (Sartorius, Göttingen, Germany) collected the virus that passed through the system. An aspirator (Ecoaspir, Kare Medical, and Analytical Devices Ltd. Co., Ankara, Turkey) was connected to the outlet of the inline filter holder to ensure a smooth flow of air through the filter. A thermometer with k-type thermocouple (CEM-613, CEM, Shenzhen, China) was used to measure air temperature at the outlet of the system.

A suspension of the SARS-CoV-2 was nebulized into the venturi injector and mixed with the air entering the electric heater. The temperature of the air increased through the heater. Since the gelatin filter's maximum working temperature was 60 °C, the silicone tubing connected to the heater's outlet was cooled with ice moulds to reduce the air temperature to below 60 °C at the filter holder. The aspirator with an airflow of 0.6 m^3/h was connected to the filter holder output and used as a vacuum pump.

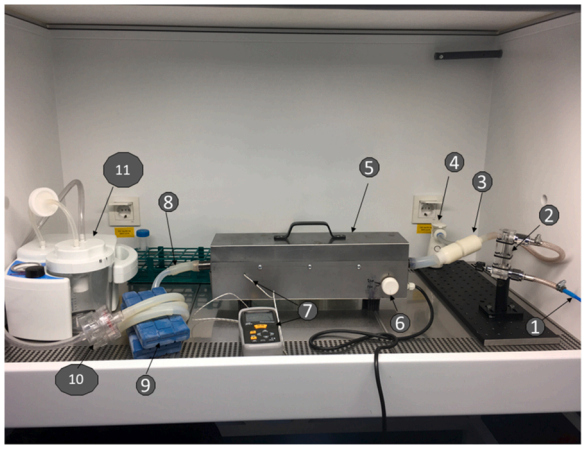

Fig. 1. 1) Silicone tubing between the air compressor and air flow meter, 2) Air flow meter, 3) Venturi injector, 4) Nebulizer, 5) Heater, 6) Heater control knob, 7) Thermometer with K-type thermocouple, 8) silicone hose, 9) Ice-moulds, 10) Gelatin filter holder, 11) Aspirator.

A detailed illustration of the electric heater is shown schematically in Fig. 2. The body of the heater was made of a metal sheet with a thickness of one mm. An electric heating coil was located perpendicular to the air flow and used as a thermal energy source in the heater. The heater volume was 4 cm× 4 cm × 15 cm = 240 cm^3. The heating coil's length was 100 cm and placed in the airway perpendicular to the air flow, with seven rows of three columns as seen in Fig. 2. The thermocouple was located at the outlet of the heater to measure the outlet temperature. The heater was covered with fibreglass slabs to ensure heat insulation, as seen in Fig. 2.

In the control experiment, the heater was off, and the compressor and the nebulizer were turned on for five minutes. The control experiments were done twice. The airflow rate was set to 0.6 m^3/h in all the experiments. In the test experiments, the compressor and the heater were turned on, and then the outlet air temperature was set to 150 ± 5 °C in the first experiment and 220 ± 5 °C in the second experiment by varying the current through the electric heating coil. Then, the nebulizer was turned on. After five minutes, the nebulizer was turned off, then the heater and the compressor were turned off. After the experiments, the gelatin filter inside the inline polycarbonate filter holder was dissolved in PBS (Phosphate Buffered Saline) at 37 °C for five minutes to harvest the virus.

3. Results

The concentration of the stock virus was 7.5 $\log_{10}$ $TCID_{50}$. The control experiments were performed twice, and the average viral load obtained in the gelatine filter was 5.5 $\log_{10}$ $TCID_{50}$. In setting the heater outlet air temperature to 150 ± 5 °C, the viral load was reduced to 2.5 $\log_{10}$ $TCID_{50}$. The reduction in the viability of the virus was 3 $\log_{10}$ or 99.9 %. At the higher temperature of 220 ± 5 °C, the virus load viability was 0.5 $\log_{10}$ $TCID_{50}$, and the reduction in the infectivity of the virus was 5 $\log_{10}$ or 99.999 %.

In the table, R is the reduction in viral load, C is an average of $TCID_{50}$ of the positive control groups, and T is the $TCID_{50}$ values of the test groups. The residence time of the air was calculated using the heater volume, V = 240 cm^3, and the air flow rate, Φ=0.6 m^3/h = 166.6 cm^3/s in $t = V/\Phi$ as 1.44 s. During this time, the air temperature increased from 20 °C to 150 °C or 220 °C.

4. Discussion

In order to limit the spread of SARS-CoV-2, liquids must be decontaminated before disposal. Therefore, as one of the alternative decontamination methods, thermal inactivation of SARS-CoV and SARS-CoV-2 in liquids has been investigated in several studies (Auerswald et al., 2021; Batéjat et al., 2021; Biryukov et al., 2021; Burton et al., 2021)

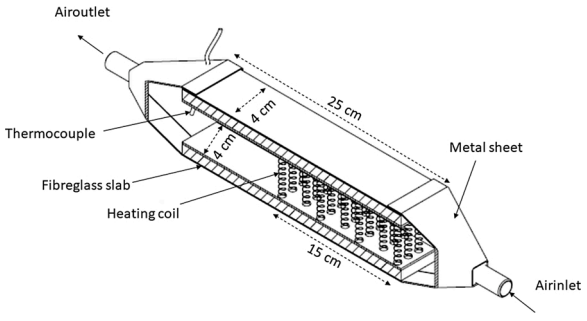

Fig. 2. Detailed schematic presentation of the heater.

However, to the best of our knowledge, there is no study to date for quantifying the inactivation of viable airborne SARS-CoV-2 exposed to high temperatures.

The inactivation of some airborne bacteria and viruses by high heat exposure has been studied previously (Grinshpun et al., 2010a,b; Lee and Lee, 2006). For example, when the exposure temperature is below 200 °C, the change in the viability of aerosolized *Bacillus subtilis* spores is negligible. At an exposure temperature above 320 °C and heat exposure duration of 0.2−0.3 s, the viability loss is 99.99 % (Grinshpun et al., 2010a). In the study of Lee et al., MS2 viruses exposed to temperatures of 90 °C and 250 °C in the time range of 0.1−1 s, viral infectivity loss was 90 % and 99.996 %, respectively. Results show that airborne SARS-CoV-2 thermal resistance is less than *Bacillus subtilis* spores and closer to MS2 virion.

An analytical model based on the rate law for a first-order reaction and the Arrhenius equation was used to determine the temperature dependence of the rate constant and estimate the time required to inactivate SARS-CoV-2 (Hessling et al., 2020; Yap et al., 2020). The rate constant $k(T)$ for thermal inactivation of both SARS-CoV-2 and SARS-CoV in a liquid or on a surface was obtained based on the Arrhenius model and linear regression for log k(T) (Hessling et al., 2020) as

$$k(T) = 10^{-\frac{5574.7}{T}+15.928} \quad (1)$$

The inactivation time of a virus at a selected temperature is defined as (Hessling et al., 2020)

$$10^{LR} = 10^{-k(T)t} \rightarrow t = \frac{LR}{k(T)} \quad (2)$$

LR is a logarithmic reduction of a virus at temperature *T* (K) within a time of *t*. Using Eq.1 and Eq.2, we calculated an inactivation time 0.320 s for 3 $\log_{10}$ reductions at 150 °C and 0.007 s for 5 $\log_{10}$ reductions at 220 °C in the viral load. The passage time of the virus in the heater is 1.44/0.320 = 4.5 times and 1.44/0.007 = 205 times longer than the calculated time needed to inactivate the virus at the temperature of 150 °C and 220 °C. The approach of using the heater outlet temperature as a reference is therefore reasonable since the estimated times for inactivation of the viruses are a fraction of a second and much smaller than the passage time for the selected temperatures. Heat exposure times for inactivation of aerosolized SARS-CoV-2′ were estimated using the inactivation data of the virus in liquids or on a surface due to the absence of the inactivation data of the aerosolized virus. Hence, the calculated inactivation times may differ slightly from the aerosolized virus's inactivation time under the same heat exposure conditions.

The gelation film collected the virus at both temperatures for 5 min during the test experiments. Before arriving at the gelation filter, the air temperature was lowered to 45 °C not to cause damage. At this temperature, the calculated relative humidity of the air just before entering the filter is approximately 29 %, based on the mixing ratio of air and nebulized water in the system. One may wonder about the inactivation of the viruses collected on the filter and its contribution to reducing the overall viral load. The LR of the contained virus by the filter can be calculated using Eqs. 1 and 2 for the temperature of 45 °C and the average exposure time of 2.5 min for both experiments. The LR is obtained as 0.0625. As seen in Table 1, log10 TCID50 values of the collected virus by the gelatin filter are 2.5 for the first experiment and 0.5 for the second experiment. Therefore, we can calculate the reduction in the viral load on the filters using

$$\frac{C}{C_0} = 10^{-n} \quad (3)$$

C values $10^{2.5}$ and $10^{0.5}$ are the viral loads at the end of the experiments performed at temperatures of 150 °C and 220 °C, respectively, and n = 0.0625. The viral load before the inactivation on the filters, C_o, was calculated using Eq. (3) as $10^{2.5625}$ and $10^{0.5625}$ for the first and second experiments, respectively. The amount of inactivated viruses on the filters due to exposure to hot air is $10^{0.0625}$ and is negligible for both experiments.

Table 1
$\log_{10}$ TCID50 values of the stock virus, control, and test groups.

TEST	Experiments	($\log_{10}$ $TCID_{50}$)	Mean	The average reduction in the viral load	
Virus Titration	Stock virus	7.5	–		
Virucidal Test (5 min at 150 °C)	Positive Control 1	5.00	5.50	R = $\log_{10}$C-$\log_{10}$ T R = 3.00	% 99.900
	Positive Control 2	6.00			
	Test 1	2.5	–		
Virucidal Test (5 min at 220 °C)	Positive Control 1	5.00	5.50	R = $\log_{10}$C-$\log_{10}$ T R = 5.00	% 99.999
	Positive Control 2	6.00			
	Test 1	0.5	–		

The flow velocity is 0.1 m/s within a 4 cm cross-section, and the Reynold number is about 250, which is laminar flow. Due to the laminar flow, the air may not be heated homogeneously to inactivate the virus. Therefore, the heat distribution of the flow through the heater needed to be validated. Additionally, the presented work is a proof-of-concept study related to the inactivation of the aerosolized SARS-CoV-2 at high temperatures and within a few seconds while passing through an electric heater. The presented method has not yet been validated in reducing viral load in a room.

A practical application of the method may be an indoor electric heater during winter. Therefore, using the device when there is no need for heating is a limitation of it. If an indoor electric heater is developed based on the presented method, it may have dual functionality as a heater and as well as purifying indoor air by inactivating airborne pathogens using the same amount of energy as a standard indoor heater. Therefore, other air purifiers such as HEPA filters and UV-C systems are not direct competitors.

We draw attention that the heater is not likely to reach every virion in the air, and only the virion in the air passing through the device is inactivated in the room. So, there may be viable airborne virions during heating in the room. Therefore, the effectiveness of the proposed system needs to be validated in reducing viral load based on the system's airflow rate, air outlet temperature, operating time, and space volume.

5. Conclusion

A one-second exposure to a temperature of 150 °C or 220 °C showed 3 log10 or 5 log10 reductions of SARS-CoV-2. The results may be applicable to future tests with indoor heaters.

Data availability

Data will be made available on request.

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Declaration of Competing Interest

The authors report no declarations of interest.

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