Environmental Science & Technology Letters

pubs.acs.org/journal/estlcu

Letter

Dry Heat as a Decontamination Method for N95 Respirator Reuse

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Cite This: https://dx.doi.org/10.1021/acs.estlett.0c00534

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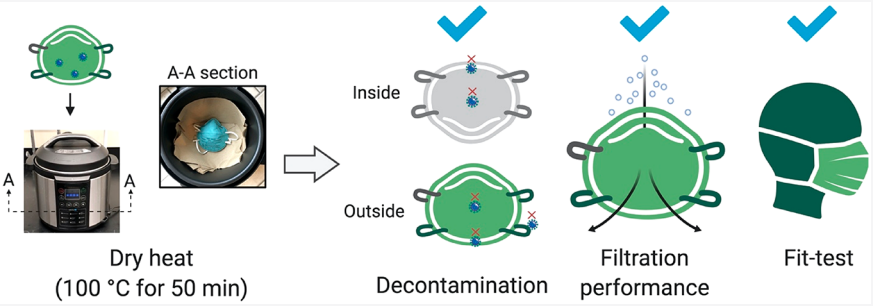

ABSTRACT: A pandemic such as COVID-19 can cause a sudden depletion of the worldwide supply of respirators, forcing healthcare providers to reuse them. In this study, we systematically evaluated dry heat treatment as a viable option for the safe decontamination of N95 respirators (1860, 3M) before their reuse. We found that the dry heat generated by an electric cooker (100 °C, 5% relative humidity, 50 min) effectively inactivated Tulane virus (TV, >5.2-$\log_{10}$ reduction), rotavirus (RV, >6.6-$\log_{10}$ reduction), adenovirus (AdV, >4.0-$\log_{10}$ reduction), and transmissible gastroenteritis virus (TGEV, >4.7-$\log_{10}$ reduction). The respirator integrity (determined on the basis of the particle filtration efficiency and quantitative fit testing) was not compromised after 20 cycles of a 50 min dry heat treatment. On the basis of these results, dry heat decontamination generated by an electric cooker (e.g., rice cookers, instant pots, and ovens) could be an effective and accessible decontamination method for the safe reuse of N95 respirators. We recommend users measure the temperature during decontamination to ensure the respirator temperature can be maintained at 100 °C for 50 min.

INTRODUCTION

An N95 respirator is an essential piece of personal protection equipment (PPE) during an outbreak of an infectious disease. Although the respirator is disposable, the high demand during a pandemic such as COVID-19 has forced healthcare providers to reuse respirators. 3M, the main respirator manufacturer, has issued four recommendations for reuse.[1] First, the decontamination should be virucidal under relevant conditions. For example, the Food and Drug Administration (FDA) requires a 6-$\log_{10}$ reduction of three non-enveloped viruses in soiling agents for respirators belonging to a single user.[2] Second, the filtration performance (filtration efficiency and breathability) should be maintained after the decontamination process. Third, the treated respirator must be leak-tight, fitting closely against the user's face without obvious gaps that permit air to enter between the respirator and the user's face. Fourth, the decontamination method must not leave residual harmful chemicals. We recommend an additional requirement that the decontamination technology should be easily accessible. Dry heat has the potential to satisfy these five requirements. The thermal treatment is effective for various pathogens.[3] Dry heat is the least likely to reduce the filtration efficiency when compared with other available decontamination methods (moist heat, ethanol, isopropanol solution, bleach, and ultraviolet).[4,5] Dry heat can also be generated by electric heating appliances (e.g., rice cookers, instant pots, and ovens) without using toxic materials. Although a body of evidence supports dry heat for respirator reuse,[3−5] most of the studies did not consider the five requirements simultaneously. In this research, we tested the viability of a commercial electric cooker for N95 respirator reuse. We conducted experiments for viral decontamination, filtration performance, and quantitative fit testing. Four viral pathogens covering a range of structures and genomes were tested. Molecular assays were applied to reveal the inactivation mechanisms. On the basis of the results, dry heat (100 °C, 5% relative humidity, 50 min) is an appropriate decontamination technology for N95 respirator reuse.

Received: July 8, 2020
Revised: July 10, 2020
Accepted: July 15, 2020
Published: July 15, 2020

MATERIALS AND METHODS

Respirator and Cooker. We used N95 respirators (1860, 3M) and an electric cooker (WM-CS60004W, Farberware), which is an inexpensive and commonly available kitchen appliance. The pot was 22 cm in diameter, 15 cm in height, and 5.7 L in volume. The surface temperatures of the pot and the respirator were monitored every 5−13 min during the dry heat treatment using an infrared thermometer (IRT205, General Tools). The temperature and relative humidity of the air inside the pot were measured using a thermo-hygrometer (A600FC, General Tools).

Testing Viruses. To fulfill the FDA requirements for viral inactivation, we used four different viruses with different virus genomes and capsid structures: human adenovirus type 2 (AdV; *Adenoviridae*, dsDNA, single-layer non-enveloped virion),[6] rotavirus OSU (RV; *Reoviridae*, dsRNA, triple-layer non-enveloped virion),[7] Tulane virus (TV; *Caliciviridae*, ssRNA, single-layer non-enveloped virion),[8] and porcine transmissible gastroenteritis virus (TGEV; *Coronaviridae*, ssRNA, single-layer enveloped virions).[9] TGEV and SARS-CoV-2 are enveloped (+)ssRNA viruses with a genome encapsulated in a nucleocapsid protein (N).[10] Details of the virus preparation methods are provided in Text S1. All experiments were replicated three times.

Decontamination Test. We performed three separate procedures to test the inactivation efficacy. First, we inoculated TV in five different locations (the inside edge, the inside center, the outside edge, the outside center, and the strap) on one whole respirator to see the effect of the inoculation site on virus inactivation efficacy. The virus suspension was mixed with artificial saliva in a 1:1 ratio, and 30 μL of this mixture was used for inoculation. Note that the volume of 30 μL is larger than the actual size of droplets released from an infected patient.[11] We applied dry heat and then cut the respirators into pieces at the inoculation sites. Second, we cut a clean respirator into 5 mm diameter pieces, inoculated them with TV, and surrounded them with a polycotton lab coat (Fisher Scientific) in the pot to simulate a case in which the dominant heat transfer method is convective heat instead of radiation heat from the interior walls of the pots. Third, we inoculated 5 mm diameter clean pieces with each of the four viruses and used dry heat for various time spans. Details are provided in Text S2.

We submerged respirator pieces in 1 mL of fresh culture medium and detached the viruses from the respirator fragments by vortexing them for 3 min and shaking them for 30 min at 450 rpm (Figure S1). The supernatant was used for the plaque assay and the molecular assays to determine the inactivation efficacy and mechanisms, respectively. We used the previously established molecular assays with a slight modification to analyze the primary structural target of TV by the dry heat treatment.[12,13] An RNase assay, a binding assay, and a two-step RT-qPCR assay were applied to examine the capsid protein integrity, binding protein integrity, and intact genomes, respectively. Details about one-step and two-step RT-qPCR are included in Text S3, as recommended by the MIQE guidelines.[14] Because the C_t values at the two consecutive dilutions that ranged from 10^2 to 10^7 PFU/mL were around −3.3, inhibition was not a concern for RT-qPCR in the dilution range.[15] The binding assay was conducted for TV solutions with infectivity values from 10^3 to 10^7 PFU/mL. This range was determined on the basis of the linear relationship (R^2 = 1.00 and slope of 0.95) between infectivity and the number of genome copies obtained by the binding results of serially diluted intact TV solutions. The two-step RT-qPCR assay was conducted for serial dilutions of TV solutions ranging from 10^2 to 10^7 PFU/mL, on the basis of the linear relationship between infectivity and the number of genome copies (R^2 = 0.98 and slope of 1.11).

Filtration Performance Test. The particle filtration efficiency of the filters was determined using charge-neutralized NaCl particles. The detailed experimental setup and procedure are provided in the Supporting Information (Text S4 and Figure S2). Briefly, a small portion (47 mm diameter) of the N95 mask fabric was cut and loaded onto a 47 mm filter holder (URG, Carrboro). A 2% NaCl solution was aerosolized using a constant output atomizer (TSI model 3076).[16] The polydisperse NaCl aerosols generated from the atomizer were first dried and charge-neutralized; they were then passed into a polypropylene chamber, which housed the filter holder. We used a condensation particle counter (CPC, TSI model 3022A; flow rate of 1.5 L/min) to measure the particle concentration before and after the test filter (i.e., a section of the mask) had been loaded in the filter holder. We tested the filters for a face velocity of 9.4 cm/s (equivalent to the NIOSH-recommended test flow rate of 85 L/min). A pressure gauge (Magnehelic 1−10 in. of water) was also connected in parallel and downstream of the filter holder using a T-connector to measure the pressure drop across the mask. The particle number concentration was measured before and after the filter holder had been connected, and the particle removal efficiency of the mask was measured by the following equation:

$$\text{particle removal efficiency (\%)} = \left[1 - \frac{\text{particle number concentration after placing the mask (no./cm}^3\text{)}}{\text{particle number concentration before placing the mask (no./cm}^3\text{)}}\right] \times 100$$

The filtration performance test was performed on each respirator after 1, 2, 3, 5, 10, and 20 cycles of dry heat decontamination.

Quantitative Fit Testing. Quantitative fit testing was performed by the Office of Occupational Safety and Health at the University of Illinois at Urbana-Champaign following the modified ambient aerosol condensation nuclei counter quantitative fit testing protocol.[17] Three respirators treated by 20 cycles of dry heat for 50 min were prepared. The testing room was filled with a NaCl aerosol, which was produced by a particle generator (8026, TSI). A test taker donned each respirator connected to a respirator fit tester (8046-T, TSI). The respirator fit tester analyzed the NaCl concentrations both in the ambient air and inside the respirator to quantify the respirator fit. The fit factor is defined as the ratio of the NaCl concentration in the ambient air to that inside the respirator. The average fit factor should be >100 for an N95 respirator throughout the following exercises: bending over for 50 s, talking for 30 s, turning one's head from side to side for 30 s, and nodding one's head up and down for 30 s.

https://dx.doi.org/10.1021/acs.estlett.0c00534
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RESULTS AND DISCUSSION

Decontamination Efficacy. The temperature of the pot surface rapidly increased to 170 °C within 5 min and then dipped to remain between 120 and 150 °C inside the pot (Figure 1a). The temperature of the cooker exterior was ∼50 °C, and the respirator temperature reached the final temperature range of 95−105 °C within 30 min. This temperature range was maintained throughout one complete cycle of dry heat treatment (50 min). The ambient air temperature was similar to that on the respirator's surface, and the relative humidity was maintained at ∼5%.

The final TV infectivity was lower than the detection limit in 50 min regardless of the inoculation site, suggesting that the entire respirator was sufficiently heated and the dry heat effectively inactivated the viruses across the entire respirator. When the respirator piece was surrounded by polycotton, the final infectivity was also lower than the detection limit (>5.2-$\log_{10}$ reduction), indicating that stacking or wrapping the respirators will not severely prevent decontamination by dry heat treatment. The dry heat also effectively inactivated the four viruses, reducing the levels of the viruses to below the detection limits in 50 min (Figure 1b). As shown in Figure 1c, a >4.3-$\log_{10}$ reduction in TV infectivity was associated with a >2.3-$\log_{10}$ reduction in the capsid protein integrity, a 0.9-$\log_{10}$ reduction in the binding protein integrity, and a 0.4-$\log_{10}$ reduction in the intact genome. These results suggest that the loss of virus infectivity was primarily due to capsid damage caused by the dry heat.

Filtration Performance and Quantitative Fit Testing. We conducted two types of experiments to prove the integrity of the respirator: filtration efficiency and pressure drop. As shown in Figure 2, the initial particle filtration efficiency of the new mask was >99% at a face velocity of 9.4 cm/s. After 20 cycles of 50 min treatments, the particle filtration efficiency was still above 95% (i.e., 97%). The pressure drop across the mask was also not significantly affected by the decontamination process, which can be seen clearly in Figure 2b. Each fit factor of the respirator treated with 20 cycles of the dry heat was 120, 141, and 156. Because the passing score is 100, all treated respirators passed the quantitative fit testing. Collectively, these results suggest that dry heat decontamination does not compromise the respirator integrity even after 20 cycles of the treatment.

Decontamination Efficacy and Mechanisms. Dry heat (100 °C for 50 min) successfully conveyed the thermal energy to the viruses, resulting in a >5.2-$\log_{10}$ reduction for TV, a >6.6-$\log_{10}$ reduction for RV, a >4.0-$\log_{10}$ reduction for AdV, and a >4.7-$\log_{10}$ reduction for TGEV. After 30 min, RV was the most resistant to dry heat among the four viruses. This is consistent with a previous study that found that RV was more heat resistant (80 °C) than TV in culture medium.[18] We expect TV, AdV, and TGEV to be inactivated as efficiently as RV after 50 min, too, assuming that the thermal inactivation kinetics follow the first-order reaction.[19]

The respirator materials were found to affect the inactivation efficacy because the decrease in infectivity after treatment for 20 and 30 min was significantly higher when viruses were inoculated on the hydrophilic surfaces ($p < 0.05$). After treatment for 40 and 50 min, the final infectivity was below the detection limit of the plaque assay. The higher inactivation efficacy of viruses inoculated on the hydrophilic surface (inside of the respirator) compared to that on the hydrophobic surface

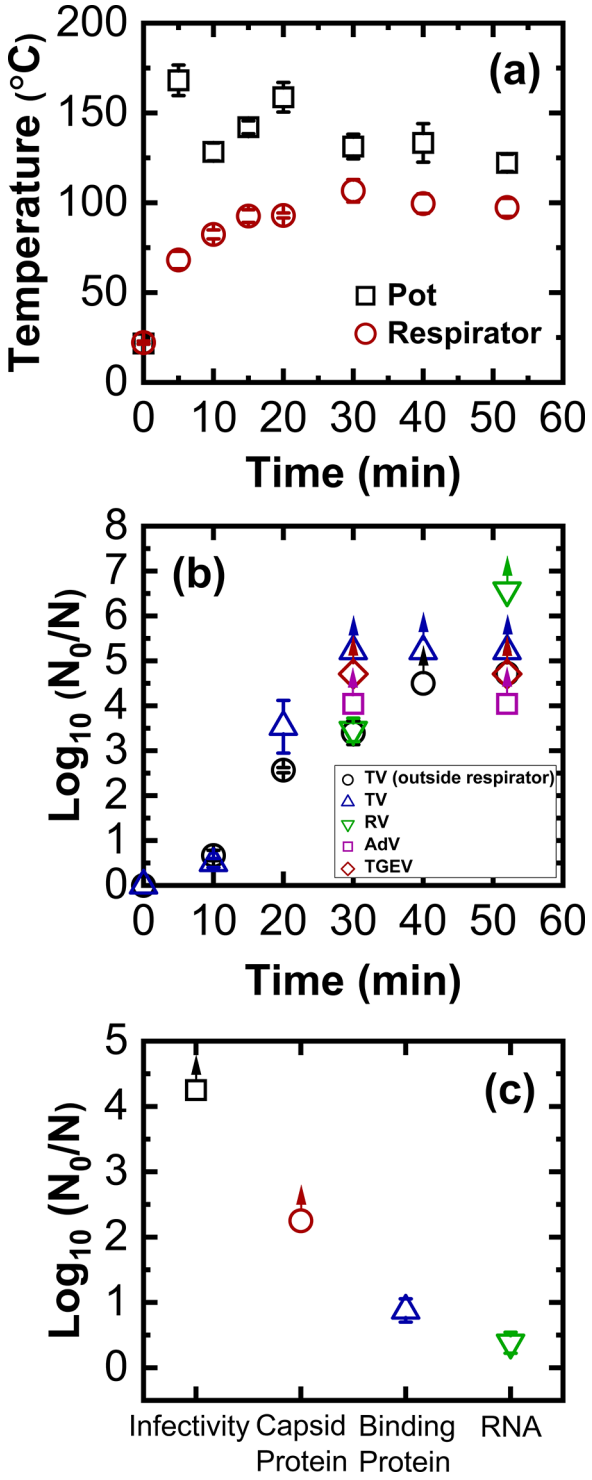

Figure 1. Effect of dry heat treatment on (a) the temperature profiles for the surfaces of the pot and the respirator and (b) the virus inactivation rates. Tulane viruses were inoculated on the hydrophobic (outside) and hydrophilic (inside) surfaces, while the other viruses were inoculated on only the hydrophilic (inside) surfaces. (c) Molecular assay results from Tulane virus samples treated with dry heat for 30 min. Reductions in virus infectivity, capsid protein integrity, binding protein integrity, and intact RNA genome were calculated by dividing the concentration of the negative control by that of the treated sample [i.e., $\log_{10}(N_0/N)$]. Arrows indicate the detection limit. The detection limit varied depending on the initial infectivity of the virus solution ($\log_{10} N_0$). All of the experiments were replicated three times.

https://dx.doi.org/10.1021/acs.estlett.0c00534
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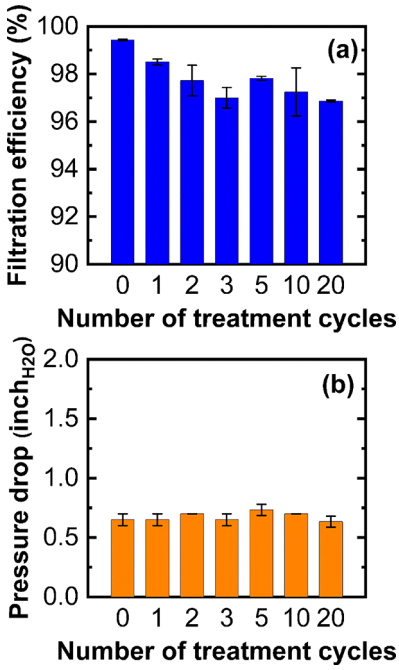

Figure 2. Effect of dry heat decontamination on (a) the particle filtration efficiency and (b) the pressure drop across the filter. All of the experiments were repeated three times.

(outside of the respirator) can be explained by how these materials held the virus solutions. After the virus solution was inoculated on these two surfaces and given 2 h to evaporate, the saliva ingredients formed a thick solid on the outside while the saliva ingredients were evenly distributed inside of the respirator (i.e., a thinner solid). This thick saliva solid is expected to protect the viruses from the dry heat.[20] Therefore, it should be noted that the mass of saliva ingredients on the respirator will affect the inactivation efficacy.

Our finding that the TV capsid protein instead of the genome was the main target of the dry heat agrees with the previous studies about non-enveloped viruses such as TV,[18] bacteriophage MS2,[21] and parvovirus.[22] Because protein denaturation follows a first-order reaction and the Arrhenius equation, virus inactivation will be significantly affected by treatment temperature and time.[3] A recent study showed that dry heat (82 °C, 30 min) using a lab oven was not enough to achieve a 3-log$_{10}$ reduction for MS2, Phi6, and murine hepatitis viruses.[23] Also, the inactivation efficacy of dry heat (100 °C, 15 min) for MS2 was no greater than a 1-log$_{10}$ reduction.[24] This result aligned with our findings that dry heat (100 °C, 10 min) inactivated TV by a factor of <1 log. However, the virus infectivity decreased rapidly to at least 3 log$_{10}$ after 30 min. Collectively, these results suggest that proper decontamination requires the optimal temperature and treatment time. The dry heat generated by the cooker (100 °C, 50 min) was the optimal condition for the inactivation of tested viruses. Because an ∼4-log$_{10}$ reduction of SARS-CoV-2 on the respirator's surface was achieved by applying dry heat (70 °C, 60 min),[25] the dry heat used in this study (100 °C, 50 min) should be adequate to inactivate SARS-CoV-2.

The respirator integrity (filtration performance and fit testing) did not degrade after 20 cycles of the dry heat treatment.[5] Note that the filtration performance and quantitative fit testing do not guarantee the respirator can be reused for 20 cycles because the respirator integrity will also be affected by the user donning and doffing the respirator.[26] Although the temperature of the respirator's surface was higher than the maximum operating temperature (50 °C) that is provided by the manufacturer,[27] the primary materials for the respirator (polyester, polypropylene, polyurethane, and polyisoprene) can withstand a temperature as high as 150 °C.[28,29] Because the temperature of the pot surface is higher than the allowable temperature for the outside surface of the respirator (polypropylene), direct contact between the respirator and the pot surface must be avoided using a towel or some other item to create a barrier and insulate the respirators. It was reported that N95 respirators partially melted when they were placed directly on metal pans in a lab oven at 100−120 °C (Isotemp 500 Series, Fisher Scientific).[30,31] We confirmed that the respirator filtration efficiency (98.5 ± 0.1%) and the pressure drop (0.7 ± 0.0 in.$_{H_2O}$) were still acceptable for the N95 respirator placed on the polycotton after the dry heat generated by the lab oven (Isotemp 650G, Fisher Scientific) set at 120 °C (the temperature of the respirator's surface was 110 °C) for 24 h.

In conclusion, dry heat treatment at 100 °C for 50 min is an appropriate method for preparing N95 respirators (1860, 3M) for reuse. Further studies of other types of respirator reuse are needed because different materials may require different temperatures and treatment times to produce the same treatment result. The heating appliances would work for respirator reuse only if they provide the proper dry heat and enough space so that 100 °C can be maintained for 50 min across the respirator. Note that temperatures of >100 °C, the dry heat could reduce the respirator integrity[30,31] while temperatures of <100 °C may require a longer treatment time to inactivate the viruses.[23,24]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.0c00534.

Detailed information about experimental methods, including virus preparation, experimental procedures for the decontamination test, the molecular assays for assessing the primary damage of the Tulane virus, the NaCl particle filtration efficiency test, and information about RT-qPCR conditions and primers, the calibration curve for virus detachment by vortexing for 3 min and shaking at 450 rpm for 30 min (Figure S1), and the experimental setup for testing the NaCl particle filtration efficiency of the respirator (Figure S2) (PDF)

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https://dx.doi.org/10.1021/acs.estlett.0c00534
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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported jointly by EPA/NIFA Grant on Water Reuse 2017-39591-27313. Its contents are solely the responsibility of the grantee and do not necessarily represent the official views of the EPA. Further, the EPA does not endorse the purchase of any commercial products or services mentioned in the publication. The authors acknowledge Dr. Hebbard, Dr. Goodly, and Mr. Neighbors (Occupational Safety and Health) for their support and feedback on this project.

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