A pandemic such as COVID-19 can cause a sudden depletion in 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 its reuse. We found that the dry heat generated by an electric cooker (100°C, 5% relative humidity, 50 min) effectively inactivated Tulane virus (>5.2-log_{10} reduction), rotavirus (>6.6-log_{10} reduction), adenovirus (>4.0-log_{10} reduction), and transmissible gastroenteritis virus (>4.7-log_{10} reduction). The respirator integrity (determined based on the particle filtration efficiency and quantitative fit testing) was not compromised after 20 cycles of 50-min dry heat treatment. Based on these results, we propose dry heat decontamination generated by an electric cooker (e.g., rice cookers, instant pots, ovens) to be an effective and accessible decontamination method for the safe reuse of N95 respirators.
Dry heat as a decontamination method for N95 respirator reuse

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Graphic TOC

Abstract

A pandemic such as COVID-19 can cause a sudden depletion in 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 its reuse. We found that the dry heat generated by an electric cooker (100°C, 5% relative humidity, 50 min) effectively inactivated Tulane virus (>5.2-log₁₀ reduction), rotavirus (>6.6-log₁₀...
reduction), adenovirus (>4.0-log\(_{10}\) reduction), and transmissible gastroenteritis virus (>4.7-log\(_{10}\) reduction). The respirator integrity (determined based on the particle filtration efficiency and quantitative fit testing) was not compromised after 20 cycles of 50-min dry heat treatment. Based on these results, we propose dry heat decontamination generated by an electric cooker (e.g., rice cookers, instant pots, ovens) to be an effective and accessible decontamination method for the safe reuse of N95 respirators.

Introduction

An N95 respirator is an essential piece of personal protection equipment (PPE) during an outbreak of infectious disease. Although the respirator is designed to be disposable, the high demand during a pandemic such as COVID-19 can force healthcare providers to reuse respirators. The primary problem with respirator reuse is that once a respirator is contaminated, it can act as a potential transmission route of pathogens to both patients and healthcare providers. The safety of healthcare providers depends on respirators being effectively decontaminated prior to reuse.\(^1\) 3M, the main respirator manufacturer, has issued four recommendations for reuse.\(^2\) First, the decontamination should be virucidal under relevant conditions. For example, the Food and Drug Administration (FDA) requires at least 3-log\(_{10}\) virucidal efficacy for multiple viruses, including coronaviruses in soiling agents (e.g., blood, mucus, or sebum).\(^3\) 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 such that there are no 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 that affect the user’s safety.
We recommend an additional requirement that the decontamination technology should be easily accessible.

Dry heat has the potential to satisfy the five requirements mentioned above. Heat is one of the most conventional disinfection technologies, so the thermal inactivation efficacies for various pathogens are known. 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 UV). In addition, dry heat can be generated by electric heating appliances (e.g., rice cookers, instant pots, and ovens) without using toxic materials. However, no experimental conclusions about dry heat have been made for N95 respirator reuse in terms of these five requirements. In this research, we conducted experiments for viral decontamination, filtration performance, and quantitative fit testing. Based on the results, we determined that dry heat is an appropriate decontamination technology for N95 respirator reuse.

Materials and method

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 pot could fit a stack of about 5 respirators while maintaining at least 3 cm between the respirators and the interior sides and lid of the pot. The surface temperatures of the pot and the respirator were monitored every 5 to 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 that included: dsDNA virus (respiratory human adenovirus type 2, single-layered non-enveloped virion), dsRNA virus (rotavirus OSU, triple-layered non-enveloped virion), 7 kb ssRNA virus (Tulane virus, surrogate for human norovirus, single-layered non-enveloped virion), and 28.5 kb ssRNA virus (porcine transmissible gastroenteritis virus (TGEV), single-layered enveloped virion). TGEV is categorized into Coronaviridae, the same family as SARS-CoV-2. It has the same viral structure and genome as SARS-CoV-2 (enveloped and (+)ssRNA virus), but it primarily infects pigs. Tulane virus, rotavirus (OSU strain), and human adenovirus type 2 belong to Caliciviridae, Reoviridae, and Adenoviridae, respectively. Details of the virus preparation methods are described in Text S1 and Table S1.

The virus suspension was mixed with artificial saliva at a 1:1 ratio before use. The artificial saliva was used as a soiling agent and prepared following ASTM E2720-16 with a slight modification (Table S2). All of the experiments were replicated three times.

Decontamination test

We performed three separate procedures to test inactivation efficacy. First, we inoculated Tulane virus 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 Tulane virus inactivation efficacy. We applied dry heat and then cut the respirators into pieces at the inoculation sites (Figure S1). Second, we cut a clean respirator into 5 mm diameter pieces, inoculated them with Tulane virus, and surrounded them with polycotton lab coat (Fisher
Scientific, USA) in the pot to simulate a case where the dominant heat transfer method is convective heat instead of radiation heat from the interior walls of the pots (Figure S2). Third, we inoculated 5 mm diameter clean pieces with each of the four viruses (Tulane virus, rotavirus, adenovirus, and TGEV) and used dry heat for various time spans. Details are described in Text S2.

We submerged respirator pieces in 1 mL of fresh culture media and detached the viruses from the respirator fragments by vortexing them for 3 min and shaking them for 30 min at 450 rpm (Figure S3). The supernatant was used for the plaque assay and the molecular assays to determine the inactivation efficacy and mechanisms, respectively. We calculated the reduction in virus infectivity by dividing the infectivity of the negative control by that of the treated sample (i.e., \( \log_{10} \left( \frac{N_0}{N} \right) \)). We used the previously established molecular assays with a slight modification to analyze the primary structural target of Tulane virus by the dry heat treatment.\textsuperscript{13,14} An RNase assay, a binding assay using magnetic beads coated with the host cell receptors, and a two-step RT-qPCR assay were applied to examine the integrity of capsid proteins, binding proteins, and viral genomes, respectively. Details of the molecular assays are explained in Text S3.

**Filtration performance test**

The particle filtration efficiency test of the filters was performed using a slightly modified version of the NIOSH 42 CFR 84 regulations.\textsuperscript{15} The detailed experimental setup and procedure are provided in the SI (Text S4 and Figure S4). Briefly, a small portion (47 mm diameter) of the N95 mask fabric was cut and loaded onto a 47 mm filter holder (URG, Carrboro, NC, USA). A solution of 2% NaCl was aerosolized using a constant output atomizer (TSI Model 3076, MN, USA).\textsuperscript{15} The polydisperse NaCl aerosols generated from the atomizer were first dried and charge neutralized; after which they were passed into a polypropylene chamber, which houses the filter holder. We
used a condensation particle counter (CPC, TSI Model 3022A; flow rate = 1.5 lpm) to measure the particle concentration before and after loading the test filter (i.e., a section of the mask) in the filter holder. We tested the filters for a face velocity of 9.4 cm/s (equivalent to NIOSH recommended test flow rate of 85 lpm). A pressure gauge (Magnehelic 1-10 inches 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 connecting the filter holder, and particle removal efficiency of the mask was measured by the following equation:

\[
\text{Particle removal Efficiency (\%)} = \left(1 - \frac{\text{particle number concentration after placing the mask (} \frac{\text{#}}{cm^3}\text{)} }{\text{particle number concentration before placing the mask (} \frac{\text{#}}{cm^3}\text{)}}\right) \times 100
\]

The filtration performance test was performed on each respirator after 1, 2, 3, 5, 10, and 20 cycles of dry heat decontamination. All the experiments were replicated three times.

Quantitative Fit testing

Quantitative fit testing was performed by the Office of Occupational Safety and Health at University of Illinois at Urbana-Champaign following the modified ambient aerosol condensation nuclei counter quantitative fit testing protocol (1910.134 App A, OSHA). The purpose was to check the overall integrity of the respirators. Three respirators treated by 20 cycles of 50 min of dry heat were prepared. The testing room was filled with a NaCl aerosol, which was produced by a particle generator (8026, TSI, USA). A test taker donned each respirator connected to a respirator
fit tester (8046-T, TSI, USA). The respirator fit tester analyzed the NaCl concentrations both in 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 higher than 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.

Results

Decontamination efficacy

The electric cooker is a self-contained cooking device, and water was not added to the cooker. The temperature of the pot surface rapidly increased to 170°C within 5 min and then dipped to remain between 120°C and 150°C inside the pot (Figure 1a). 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 about 5%.

The dry heat effectively inactivated the four viruses, reducing the viruses to below the detection limits in 50 min (Figure 1b). As shown in Figure 1c, >4.3-log_{10} reduction in Tulane virus infectivity was associated with >2.3-log_{10} reduction in the capsid protein, 0.9-log_{10} reduction in the binding protein, and 0.4-log_{10} reduction in the genome. Thus, the dry heat primarily destroyed the capsid proteins of Tulane virus. The Tulane virus infectivity was lower than the detection limit in 50 min regardless of the inoculation sites or having a polycotton surrounding. This suggests that thermal energy would be effectively transferred to the viruses if respirators were stacked or wrapped. On the other hand, for treatment times of 20 and 30 min, the inactivation rate was higher
when the viruses were inoculated on the hydrophilic surface (p<0.05). This can be explained by how different respirator materials held the virus solution. When the viruses were inoculated, the testing solution was absorbed by the inside of the respirator that faces the user (the hydrophilic surface). At the same time, a droplet was formed on the outside of the respirator that faces the user’s surroundings (the hydrophobic surface). After the droplet evaporated, the virus and saliva were evenly distributed inside of the respirator. In contrast, there was a high concentration of saliva remaining on the outside of the respirator, which could shield the viruses from the dry heat.
Figure 1. Effect of dry heat treatment on (a) temperature profiles for the surfaces of the pot and the respirator and (b) virus inactivation rates. Tulane viruses were inoculated on the hydrophobic (outside) and hydrophilic (inside) surfaces, while the other viruses were inoculated only on the hydrophilic surfaces. (c) Molecular assay results from Tulane virus samples treated by the dry heat for 30 min. Arrows indicate the detection limit. The detection limit varied depending on the initial infectivity of the virus solution ($\log_{10} N_0$).
Filtration performance

The integrity of filtration performance is crucial for respirator reuse. 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, 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, as evident from Figure 2b. Collectively, these results suggest that the dry heat decontamination does not compromise the integrity of the filter material even after 20 cycles of the treatment.
Figure 2. Effect of dry heat decontamination on (a) the particle filtration efficiency and (b) the pressure drop across the filter. All the experiments were repeated thrice.
Quantitative fit testing

The respirator treated with 20 cycles of the dry heat had an average fit factor of 139±18, which is higher than the fit factor required of the N95 respirator. In addition, no visible deformations (such as burning signs, nose form detachment, loss of elasticity in the band, or deformation of the entire shape) except for ink spread (Figure S5) were noticed on the respirator after 20 cycles of dry heat.

Discussion

Our experiments simulated droplets or aerosols of an infected patient’s saliva depositing on a healthcare provider’s respirator and then evaporating. The inside of the respirator could also be contaminated by the viruses while handling the respirator with contaminated hands. The dry heat generated by the cooker was confirmed to satisfy the five requirements for respirator reuse (decontamination efficacy, filtration performance, fit testing, no toxic residual chemicals, and accessibility).

The dry heat (100°C for 50 min) successfully conveyed the thermal energy to the viruses and denatured capsid proteins, resulting in >5.2-log_{10} reduction for Tulane virus, >6.6-log_{10} reduction for rotavirus, >4.0-log_{10} reduction for adenovirus, >4.7-log_{10} reduction for TGEV. Since the protein denaturation follows first-order reaction and Arrhenius equation, the virus inactivation will be significantly affected by treatment temperature and time.\(^4\) A recent study showed that dry heat (82°C, 30 min) using a lab oven was not enough to achieve 3-log_{10} reduction of MS2, Phi6, and murine hepatitis virus.\(^17\) Also, the inactivation efficacy of dry heat (100°C, 15 min) for MS2 was no greater than 1-log_{10} reduction.\(^18\) This result aligned with our findings that the dry heat (100°C) for 10 min inactivated Tulane virus by a factor of less than 1-log. However, the virus infectivity reduced rapidly by a factor of greater than 3-log_{10} after 30 min. Those results
collectively can be explained by the fact that the optimum temperature and time should be provided for the proper decontamination. The dry heat generated by the cooker (100°C) for 50 min was the optimal condition for the inactivation of tested viruses. Because about $4 \log_{10}$ reduction of SARS-CoV-2 on the respirator’s surface was achieved by applying dry heat (70°C for 60 min), the dry heat used in this study (100°C for 50 min) should be adequate to inactivate SARS-CoV-2.

The respirator integrity (filtration performance and fit testing) was not degraded by 20 cycles of the dry heat treatment. Although the temperature of the respirator’s surface was higher than the maximum operating temperature (50°C) that is provided by the manufacturer, the primary materials for the respirator (polyester, polypropylene, polyurethane, polyisoprene) can withstand a temperature as high as 150°C. Note that the temperature of the pot surface is higher than the allowable temperature for the outside surface of the respirator (polypropylene), so 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 melt when subjected to dry heat generated by a lab oven at 100-120°C (Isotemp 500 Series, Fisher Scientific). In these studies, however, the respirators were placed directly on the metal pan. We confirmed that the respirator filtration efficiency (98.5±0.1%) and the pressure drop (0.7±0.0 inch H2O) were still acceptable for the N95 respirator 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 hours.

The dry heat can be produced using readily available heating appliances. The respirator can also be reused after dry heat treatment without further treatment because no toxic chemicals were involved. Given the filtration efficiency of the respirators after being treated by the dry heat generated by the cooker (20 cycles) and the lab oven (24 hours), we believe that any device
providing dry heat and holding the respirator temperature at 100°C for 50 min would work for respirator reuse. Note that at temperatures higher than 100°C, the dry heat could reduce the respirator integrity while temperatures lower than 100°C may require a longer treatment time to inactivate the viruses.

In conclusion, dry heat treatment of 100°C for 50 min is an appropriate method for preparing N95 respirators (1860, 3M) for reuse. Further studies for other types of respirator reuse are needed because different materials may require different temperatures and treatment times to produce the same treatment result.

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Supporting information for

Dry heat as a decontamination method for N95 respirator reuse

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2 Tables
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Text S1. Testing virus preparation

Tulane virus was received from Cincinnati Children’s Hospital Medical Center and rotavirus OSU strain was obtained from ATCC (VR-892). The MA104 cell line was used to propagate Tulane virus and rotavirus. The culture medium for the MA104 cells was prepared by mixing 1X minimum essential medium (MEM; Thermo Fisher Scientific, MA, USA) with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, MA, USA), 1X antibiotic-antimycotic (Thermo Fisher Scientific, MA, USA), 17 mM of NaHCO₃, 10 mM of HEPES, and 1 mM of sodium pyruvate. MA 104 cells with 80-90% confluency were washed with PBS and inoculated with Tulane virus or rotavirus (OSU strain) in 175 cm² flasks at a multiplicity of infection (MOI) of 0.1. The inoculated cells were incubated at 37°C in a 5% CO₂ environment for an hour with gentle shaking every 10 to 15 min. Then, 20 mL of the culture medium were added to each flask. For Tulane virus, FBS was added to the culture medium at a final concentration of 2%. For rotavirus (OSU strain), trypsin was added to the culture medium at a final concentration of 10 µg/mL while FBS was not added. The infected flasks were incubated until an 80% cytopathic effect (CPE) was reached. The viruses were harvested after three freeze-thaw cycles. Both viruses were purified in 1 mM NaCl and 0.1 mM CaCl₂ solution using an ultracentrifuge (Optima XPN-90 Ultracentrifuge, Beckman Coulter, CA, USA). The ultracentrifuge was run at 1000 rpm (116 g) at 4°C for 5 min followed by 36000 rpm (150700 g) at 4°C for 3 hours. The final concentrations of Tulane virus and rotavirus were about $10^7$ and $10^8$ PFU/mL, respectively. The decontamination efficacy of both viruses was determined by plaque assay using the MA104 cell line. The incubation time for the plaque assay was 2 and 3 days for Tulane virus and rotavirus, respectively. Detailed information is described in our previous work.²,³
Adenovirus was obtained from ATCC (VR-846). They were propagated in A549 cells using Ham F-12 media with 10% FBS (Thermo Fisher Scientific, MA, USA) and 1X antibiotic-antimycotic (Thermo Fisher Scientific, MA, USA). The adenovirus was purified in 1X PBS (Thermo Fisher Scientific, MA, USA) using the ultracentrifuge and had a final infectivity of about $10^6$ PFU/mL. A volume of 2 mL of overlay solution for the plaque assay was prepared by mixing 1.31 mL of 2X MEM, 0.5 mL of 1% agarose solution, 0.1 mL of FBS, 0.05 mL of 15 mM HEPES, 0.03 mL of 7.5% sodium bicarbonate, and 0.01 mL of 100X antibiotic-antimycotic. The incubation time for the plaque assay was 5 days. Detailed information is described in our previous work.4

Transmissible Gastroenteritis Virus (TGEV) was obtained from the Veterinary Diagnostic Laboratory at the University of Illinois at Urbana-Champaign. Swine testis (ST) cells were used as a host cell for the virus to grow in and for the plaque assay. The same culture medium described for Tulane virus was also used for the ST cells. TGEV was harvested in the culture medium by centrifugation at 2000 rpm (556 g) for 10 min (Sorvall Legend RT Plus, Thermo Fisher Scientific, MA, USA), followed by filtration through a 0.45 μm filter (Millipore Sigma, MA, USA). The infectivity of TGEV was determined by the plaque assay; ST cell monolayers were prepared in 6-well plates (USA Scientific, FL, USA). The 750 μL of virus solution was inoculated to the cells followed by incubation at 37°C with 5% CO$_2$ for 60 min. The virus solution was replaced with 2 mL of the MEM containing 1% agarose, 7.5% sodium bicarbonate, 15 mM HEPES, and 1X antibiotic-antimycotic. The overlay was solidified at 4°C for 20 min followed by the incubation at 37°C with 5% CO$_2$ for 4 days. The cellular monolayers were fixed with 10% formaldehyde for 1 hour. The plaques were visualized after the fixed cells were dyed with 0.05% crystal violet in 10% ethanol for 20 min. The initial infectivity of TGEV solution was about $10^6$ PFU/mL.
Table S1. Comparison of testing viruses and SARS-CoV-2

| Viruses                        | Family          | Primary host species | Genome             | Structure        |
|--------------------------------|-----------------|----------------------|--------------------|------------------|
| Tulane virus                   | Caliciviridae   | Rhesus macaques      | (+) ssRNA (7 kb)   | Non-enveloped    |
| Rotavirus (OSU strain)         | Reoviridae      | Pigs                 | Seg. dsRNA (19 kb) | Non-enveloped    |
| Human adenovirus type 2        | Adenoviridae    | Human                | dsDNA (30 kb)      | Non-enveloped    |
| Transmissible gastroenteritis virus | Coronaviridae | Pigs                 | (+) ssRNA (29 kb)  | Enveloped        |
| SARS-CoV-2                     | Coronaviridae   | Human                | (+) ssRNA (29 kb)  | Enveloped        |
### Table S2. Composition of artificial saliva

| Reagent                           | Amount  |
|----------------------------------|---------|
| CaCl\(_2\) \cdot H\(_2\)O       | 0.13 g  |
| NaHCO\(_3\)                      | 0.42 g  |
| NH\(_4\)Cl                        | 0.11 g  |
| NaCl                             | 0.88 g  |
| KCl                              | 1.04 g  |
| (Porcine gastric) Mucin           | 3.00 g  |
| Water                            | 1000 mL |
Figure S1. Experimental process to study the effect of the inoculation site on inactivation efficacy.
Figure S2. Experimental setup for Tulane virus inactivation where the dominant heat transfer method was convective heat instead of radiation heat from the interior walls of the pots. (a) Empty cooker, (b) A respirator piece on top of a paper towel and a polycotton lab coat, (c) Another lab coat and a paper towel over the respirator piece, and (d) closed cooker.
Text S2. Experimental procedures for decontamination test

We followed three different procedures to test (1) the effect of the inoculation site on inactivation efficacy using Tulane virus, (2) the effect of heat transfer method on inactivation efficacy using Tulane virus, and (3) the inactivation efficacy of dry heat over treatment time for each surrogate virus. (1) We inoculated each respirator with five separate 30 µL droplets of the Tulane virus and saliva mixture in five different locations: the inside edge, inside center, the outside edge, outside center, and the strap. The respirator was left in a biosafety cabinet until the testing solution had thoroughly evaporated (about 2 hours). We placed the contaminated respirator in the center of the electric cooker on top of paper towels so that the respirator was 3 cm above the bottom surface of the pot. These paper towels prevented direct contact between the respirator and the pot’s hot surface. The respirator was subject to one 50-min cycle of 100°C dry heat. We then cut the treated respirator into 5 mm diameter pieces and submerged each in 1 mL of fresh culture medium. (2) We cut a clean respirator into 5 mm diameter pieces. We inoculated these pieces with 30 µL droplets of Tulane virus and saliva mixture, left the droplets to evaporate, and then wrapped the inoculated respirator pieces in a paper towel. We lined the interior of the pot with layers of polycotton fabric, placed the paper-towel-wrapped inoculated pieces in the center of the pot, and then covered the pieces with another layer of polycotton. The polycotton lining simulates respirators being stacked or enclosed in a bag so the dominant heat transfer method is convective heat instead of radiation heat from the interior walls of the pots. After the dry heat application, we added each piece to 1 mL of fresh culture medium. (3) For each of the four viruses (Tulane virus, rotavirus, adenovirus, and TGEV), we inoculated 5 mm diameter pieces of a clean respirator with 30 µL droplets of the virus and saliva mixture. After being left to evaporate in the biosafety cabinet, the inoculated respirator pieces were placed on paper towels in the electric cooker and subjected
to one 50-min cycle of 100°C dry heat. We then submerged each piece in 1 mL of fresh culture medium.

We detached the viruses from the respirator fragments by vortexing them in the culture medium for 3 min and shaking them for 30 min at 450 rpm (Figure S3). We followed the same procedure for the negative controls except that they were left in the biosafety cabinet instead of the electric cooker for the same amount of time as the dry heat treatment. The supernatant was used for the plaque assay and the molecular assays to determine the inactivation efficacy and mechanisms, respectively. We calculated the reduction in virus infectivity by dividing the infectivity of the negative control by that of the treated sample (i.e., log_{10}(N_0/N)). We used the three molecular assays with a slight modification to analyze the primary structural target of Tulane virus by the dry heat treatment.^2^ An RNase assay, a binding assay, and a two-step RT-qPCR assay were developed to examine the integrity of capsid proteins, binding proteins, and viral genomes, respectively (Text S3).
Figure S3. Calibration curve for virus detachment by 3 min vortex and 30 min shaking at 450 rpm. The detachment efficiencies were calculated by dividing the loaded virus from retrieved virus. The detachment efficiencies were not significantly different from inside and outside of the respirator pieces (p>0.05).
Text S3. Molecular assays to determine the primary damage of Tulane virus

The two-step RT-qPCR assay was designed to quantify intact genomic RNA of Tulane virus. This assay consisted of RT-PCR which synthesized cDNA covering 80% of the genomic RNA and qPCR which quantified the cDNA. We hypothesize that the viruses that had intact genomes in the range of the template for the cDNA will be quantified by this assay. The RNA was extracted from the viruses using QIAmp Viral RNA Mini Kit (Qiagen, Germany) following the manufacturer’s protocol. The cDNA was synthesized using (ProtoScript First Strand cDNA Synthesis Kit, New England BioLabs, USA) by the reverse primer which was designed to cover 5534 bp of the genomic RNA. Finally, the cDNA was quantified by qPCR (PowerUp SYBR™ Green Master Mix, Applied Biosystems, USA).

The RNase assay was developed to examine the integrity of capsid proteins. The RNase (A/T1 mix, Thermo Fisher Scientific, USA) was incubated with the viruses at 37°C for 30 min. We assumed that the RNase would be able to penetrate the damaged capsid and degrade the RNA if the capsid proteins were damaged. RNase inhibitor (SupeRNase inhibitor, Sigma Aldrich, USA) reacted with the RNase treated solution at room temperature for 30 min to inhibit the RNase activity. The remaining intact RNA was quantified by RT-qPCR, which represented the integrity of capsid proteins.

The binding assay measures the integrity of binding proteins. Magnetic beads (MagnaBind carboxyl-derivatized beads, Thermo Fisher Scientific, USA) loaded by porcine gastric mucin (Sigma Aldrich, USA) were mixed with the virus solution. The viruses with intact binding proteins were bound to the magnetic beads while the viruses that lost binding ability were washed out. The viruses bound to the magnetic beads were quantified by one-step RT-qPCR (iTaq universal SYBR
green reaction mix, Bio-Rad Laboratories, USA) following the manufacturer’s protocol. Detailed information for the two-step RT-qPCR, RNase, binding assay including reagent amount, reaction time, PCR cycles, and primers are described in our previous work.\textsuperscript{2,3}
Text S4. NaCl Particle Filtration Efficiency Test

A schematic of the particle filtration testing design used in this study is shown in Figure S4. We built a polypropylene chamber with various fittings and valves to control the aerosol concentration inside the chamber. The chamber inlet valve was connected to an aerosol generator (TSI Constant Output Atomizer Model 3076). The atomizer was filled with 2% NaCl solution (which is commonly used for measuring the penetration efficiency of N95 masks\textsuperscript{5} in Milli-Q water to generate polydisperse particles (10-800 nm) at a relatively constant rate. The count median diameter of the droplets generated by the atomizer is expected to lie between 80 and 150 nm.\textsuperscript{6} Generation and Evaluation of Monodisperse Sodium Chloride and Oleic Acid Nanoparticles.\textsuperscript{7} At the inner roof of the chamber, a small fan was installed to mix the air and thus minimize spatial heterogeneity of the particle concentration inside the chamber. A vent on the roof was also provided to connect it to the compressed air, which was used to dilute the concentration of the particles inside the chamber. The aerosols generated from the atomizer were first dried by passing it through a custom-built diffusion dryer (22 in. long and 3 in. diameter tube with a concentric meshed tube for airflow), filled with 2 mm – 4 mm silica gel. The dry aerosols were then passed through a custom-built aerosol neutralizer (1” diameter and 10” long stainless steel tube with 4 Staticmaster® 2U500, 3” Ionizing Cartridges glued inside it\textsuperscript{7}) to neutralize excess charge on the aerosols’ surface. A conductive tubing was passed through the chamber and connected to a particle counter (Condensation Particle Counter, CPC, TSI, Model 3022A; flow rate = 1.5 lpm) to measure the particle concentration. Thus, a steady-state concentration of the aerosols (~45,000 particles/cm\textsuperscript{3}) was maintained inside the chamber. A small circular section of the mask was loaded into a 47 mm filter holder (URG, Carrboro, NC, USA) and air was drawn at a specific flow rate, measured by an inline flow meter (4-50 slpm; Dwyer Instruments, MI, USA) using a vacuum line.
The surface area of the N95 mask was measured manually (~150 cm$^2$) to calculate face velocity for the NIOSH recommended flow rate (i.e. 85 lpm). The face velocity for this recommended flow rate is 9.4 cm/s. Since, we used only a small section (47 mm diameter) of this mask, we drew only 10 lpm through the filter holder, which yielded an equivalent face velocity of 9.4 cm/s. Out of the total flow through the filter, CPC used 1.5 lpm, while the rest was by-passed through a T-connector. A pressure gauge (Magnehelic 1-10 inches of water) was also connected in parallel, right downstream of the filter holder using a T-connector to measure the pressure drop. The particle number concentration was measured before and after connecting the filter holder, and particle removal efficiency of the mask was measured by the following equation:

\[
\text{Particle removal Efficiency (\%)} = \left( 1 - \frac{\text{particle number concentration after placing the mask} \ (\#/\text{cm}^3)}{\text{particle number concentration before placing the mask} \ (\#/\text{cm}^3)} \right) \times 100
\]

Note, the NIOSH testing protocol recommends performing the filtration tests until the respirator reaches a loading of 200 mg NaCl (this takes around 90-100 min); however, in our current study we stopped the testing once a constant particle filtration value was obtained (10 - 15 min of total sampling time). We assume this reduced sampling time would not significantly influence our results based on several past studies showing that the filtration efficiencies obtained by measuring initial penetration (average of the first min) of N95 masks were similar to the penetration levels obtained at full loading conditions (i.e. 200 mg). NIOSH recommends N95 masks should not exceed peak air flow resistance of 35 mm (1.37 inches of water). Here, in addition to the particle
filtration efficiency we also report the pressure drop across the filter after every cycle of rice cooker decontamination to observe any effect on the inhalation resistance.

Figure S4. Experimental setup for testing the NaCl particle filtration efficiency of the respirator.
Figure S5. Respirator appearance (a) without dry heat treatment and (b) 20 cycles of dry heat treatments.
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