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Major Article

Decontamination of surgical face masks and N95 respirators by dry heat pasteurization for one hour at 70°C

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Key Words:
N95 face mask
Personal protective equipment
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Background: The need for protective masks greatly exceeds their global supply during the current COVID-19 pandemic.
Methods: We optimized the temperature used in the dry heat pasteurization method to destroy pathogens and decontaminate masks while retaining their filtering capacity.
Results: The current study showed that dry heat at both 60°C and 70°C for 1 hour could successfully kill 6 species of respiratory bacteria and one fungi species, and inactivate the H1N1 indicator virus. After being heated at 70°C for 1, 2, and 3 hours, the N95 respirators and surgical face masks showed no changes in their shape and components. The filtering efficiency of bacterial aerosol for N95 respirators were 98%, 98%, and 97% after being heated for 1, 2, and 3 hour, respectively, all of which were over the 95% efficiency required and similar to the value before being heated (99%). The filtering efficiency for surgical face masks was 97%, 97%, and 96% for 1, 2, and 3 hours of heating, respectively, all of which were also similar to the value before being heated (97%).
Conclusions: This method can be used at home and can significantly resolve the current shortage of masks.
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The coronavirus disease 2019 (COVID-19), caused by the RNA virus SARS-CoV-2, has become a respiratory disease pandemic posing serious public health risk. The WHO updates from March 31, 2020, reported 750,890 cases and 36,405 deaths in more than 200 countries.[1] SARS-CoV-2 is highly contagious, with an R_0 between 2 and 3.[2] As a large part of the global population is threatened by COVID-19, providing timely protection to the vast number of susceptible individuals in an economical way is extremely important.

Although COVID-19 is an emerging disease and studies on its transmission and prevention are evolving, previous studies on H1N1 influenza, which is also caused by an RNA virus, have indicated that the influenza virus subtype H1N1 can be transmitted by coughing or sneezing, with infectious particles of variable sizes ranging 0.1μm-100μm that may be inhaled by susceptible individuals.[3] Physical shields between the transmission source and susceptible people are the available preventive measures during the early phase of a respiratory pandemic. Common physical shields include preventive surgical face masks for most people and N95 respirators for hospital workers.[4] These personal protective equipments (PPEs) are designed to block airborne particles ranging in size from 0.1 to 10.0 μm. Theoretically, N95 respirators can filter 95% of circulating particles from the ambient air, which dramatically reduces the air load of viruses and the risk of viral infection. To date, WHO and American CDC recommend face masks or face coverings to achieve source control and prevention of COVID-19.[5,6]

As people globally face the threat of COVID-19, the need for protective masks greatly exceeds the global supply. The global COVID-19 pandemic will result in a long-term shortage of PPE, which has already become one of the most urgent challenges to our collective ability to save lives. Therefore, finding methods to reuse the masks is important and effective decontamination of PPE could be a useful strategy in this scenario. Decontamination can be achieved by both chemical and nonchemical methods; non-chemical methods include

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the use of ultraviolet light and heat. Heat treatment is more suitable for the decontamination of masks at home. Herein, we aimed to optimize the temperature of dry heat pasteurization to achieve efficient decontamination of masks by killing the pathogens, while retaining the filtering capacity of the masks.

METHODS

Experiment design

We applied dry heat at 60°C and 70°C for 1 hour to used masks. Then, we assessed the extent of decontamination via the sterility test for 7 pathogenic bacteria and the inactivation test of H1N1 indicator virus with hemaglutination (HA) assay. We also conducted fit test and filtering efficiency test using bacteria in aerosols for the heat pasteurized N95 respirators and surgical face masks.

Bacterial strains and sterility test

To assess the decontamination effect, we used 7 bacterial/fungal strains: *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC25923), *Pseudomonas aeruginosa* (ATCC27853), *Klebsiella pneumonia* (ATCC70063), *Acinetobacter baumannii* (ATCC17978), *Corynebacterium pseudodiphtheria* (ATCC10701), and *Candida albicans* (ATCC10231). These strains were cultivated according to ATCC protocols and were prepared in a saline suspension of 10^5 cells/mL. We used TK-3 microbial aerosol generator (Pusen, Changzhou, China) in a Class 1000 clean booth. The microbial aerosol generator was adjusted to have a flow volume of 5 L/min and a spray pressure of 2 kg/cm^2 to produce aerosols from the bacterial suspension. Then, the aerosol air containing bacteria was pumped through surgical masks (Medicom Co., Ltd, Shanghai, China) and N95 respirators (3M 1860 type for medical use) respirators by the airborne microbe sampler (Anderson cascade impactor, Copley Scientific Ltd, Nottingham, UK) for 5 minutes at a flow speed of 28 L/min to inoculate the masks. Two of the inoculated masks for each bacteria strain were placed in a steel box and heated at 60°C and 70°C for 1 hour in an electric oven, respectively. The third mask was used as the control without decontamination to count the inoculated bacteria by the plate count method. From the heated masks, 25 cm^2 of inoculated areas were cut into pieces and placed in brain heart infusion broth to cultivate overnight for sterility tests.

Inactivation of the indicator virus (H1N1)

We used the H1N1 strain (A/Zhejiang/1/2009[H1N1]) as the indicator virus. The H1N1 viruses were propagated in Madin-Darby Canine Kidney (MDCK) cells cultured in OptiPRO serum-free medium (Gibco, Carlsbad, CA) containing 4 mM/L Glutamine (Gibco, Carlsbad, CA) and 0.5% penicillin/streptomycin solution (Sigma), as previously described.[7] All MDCK cells were cultured in a 5% CO_2 humidified incubator. Virus titers were determined by the 50% tissue culture infective dose ($TCID_{50}$) assay. The infected MDCK cell density was adjusted to 10^6 cells/mL. Three pieces of a mask were taken and inoculated with the cell suspension infected by H1N1. Two 25 cm^2 piece was heated at 60°C or 70°C for 1 hour respectively. An unheated piece was used as the control. Then, the MDCK cell suspension (10^5 cells/mL) was seeded with each of these pieces. The MDCK cell culture supernatants were collected for the HA assay,[8] and the HA titer was determined as the highest dilution of the supernatants showing complete agglutination on the bottom of the well.

Fit testing of N95 respirators after being heated

Every 2 N95 respirators that were not inoculated were heated at 70°C for 1, 2, and 3 hours in an electric oven, respectively. The N95 respirators were first assessed for the influence of heat on their physical features, such as shape and components of the mask, and were evaluated by the fit test. We used the 3M Qualitative Fit Test Apparatus FT-30 to assess the fit of N95 respirators after being heated. This test meets the performance criteria for fit testing respirators under the current OSHA Standard for Respiratory Protection: 29 CFR 1910.134.[9] The test was performed to assure that the person undergoing the fit test can detect the bitter taste of the test solution, even at very low levels.

Measurement of filtering efficiency of pathogens in aerosols

The study assessed the filtration efficiency through measuring the filtration rate of live bacteria in aerosols, which is different from the standard method that measures particles. We designed a test to assess the efficiency of the heat pasteurized masks to filter bacteria in aerosol. Briefly, a saline suspension of *E. coli* cells (10^5 cells/mL) used to generate aerosols, as explained already. The aerosols were pumped through the masks for 5 min and were simultaneously sampled by the Anderson 6-stage sieve airborne microbe sampler that contained six petri dishes, as mentioned above. The air that did not pass through the masks was used as the control. Bacterial colonies on the petri dish were counted after 48 hour cultivation. The ratios of filtering efficiency were calculated by comparing the numbers of bacterial colonies in the air passing through the masks with that in the air that did not pass through.

RESULTS

This study showed that dry heat at both 60°C and 70°C for 1 hour could successfully kill 7 types of bacteria as well as inactivate the H1N1 virus (Table 1).

After being heated at 70°C for 1, 2, and 3 hours, the N95 respirators and surgical face masks showed no changes in their shape and components. The filtering efficacies of the N95 respirators for bacterial aerosols were 98%, 98%, and 97% after 1, 2, and 3 hours of heating, respectively; all of these efficacies were over the 95% requirement for bioparticle isolation and similar to the efficacies of the masks before

Table 1
Number of bacterial colonies inoculated on the masks and sterility test results and H1N1 titration

Strain	Controls for bacteria and H1N1 inoculated on 25 cm^2 of masks	Disinfection at 60°C	Disinfection at 70°C
Escherichia coli	3500	Sterile	Sterile
Staphylococcus aureus	4500	Sterile	Sterile
Pseudomonas aeruginosa	4100	Sterile	Sterile
Klebsiella pneumonia	6200	Sterile	Sterile
Acinetobacter baumannii	3600	Sterile	Sterile
Corynebacterium pseudodiphtheriticum	3200	Sterile	Sterile
Candida albicans	3400	Sterile	Sterile
H1N1 virus (Titration)	1:320	<1:20	<1:20

Note: The bacterial numbers of control are colonies/25 cm^2 of mask surface. The virus amount is titration.

being heated (99%). The filtering efficacies of the surgical face masks were 97%, 97%, and 96% after being heated for 1, 2, and 3 hours, respectively, and were similar to their corresponding efficacies before being heated (97%).

DISCUSSION

During a pandemic like COVID-19, the urgent shortage of masks puts billions of people at risk. A safe and convenient decontamination method to decontaminate N95 respirators and surgical face masks that are generally disposable faces many challenges, such as maximally retaining the filtration efficiency of masks and effectively decontaminate various types of pathogens with distinct resistance to decontamination method. This study applied 8 bacterial and viral strains that cover a wide range of common respiratory pathogens to evaluate the decontamination effect of dry heat at 70°C for 1 hour. We proved the dry heat method could achieve a decontamination effect while retain the filtration efficiency of surgical face masks and N95 respirators.

Chemicals are not suitable for mask disinfection because of residual chemicals that may be toxic and carcinogenic. To tackle the current shortage of protective masks, the Dutch National Institute for Public Health and the Environment conducted a pilot study and developed a method to obtain reprocessed face masks with acceptable quality.[10] This study showed that FFP2 face masks retained their shape and ability to filter particles after sterilization by a short hydrogen peroxide process once or twice. However, this procedure is not suitable for home applications because of the need for special devices and chemicals. Among the various physical methods, heat can be used for mask disinfection.

Despite wet heat is often used to sterilize hospital materials because of its excellent penetration,[11,12] the higher temperature and steam may affect the filtering efficiency of masks more than dry heat. The comparison between 2 methods for decontamination of various types of masks is necessary in future studies. In addition, UV light is generally used to disinfect material surfaces and water. Therefore, dry heat may be more appropriate for mask decontamination. Moreover, the popular use of oven at home makes dry heat convenient and economic. The major concern over the effectiveness of dry heat compared to wet heat is lower penetration of dry heat. This concern can be overcome because the masks are thin (usually 3 mm) and porous, which ensures high penetration, as demonstrated by our study; we placed 5 masks in a steel box and achieved sterility. Another crucial advantage of dry heat is the temperature used is high enough to inactivate pathogens but at the same time, retains the filtering capacity of the masks. Generally, 60°C-70°C for over 30 minutes is an optimal temperature for pasteurization of pathogens in food and vaccines.[13] We used 60°C and 70°C to assess the decontamination effect and evaluate the retention of the mask function. Seven types of common respiratory pathogens, including Gram-positive bacteria (ie, *Staphylococcus aureus* and *Corynebacterium pseudodiphtheria*), Gram-negative bacteria (ie, *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Acinetobacter baumannii*), and the fungus *Candida albicans,* were killed both at 60°C or 70°C for 1 hour, indicating the dry heat can kill a wide range of pathogenic bacteria. In practice, 70°C is selected for higher reliability. We also used the H1N1 strain as the indicator virus to assess decontamination. The H1N1 virus is an RNA-enveloped virus similar to SARS-CoV-2 that spreads among humans via the respiratory tract. Therefore, it is theoretically feasible to use H1N1 as the indicator virus for assessment of the decontamination effects on respiratory viruses. Previous studies proved that incubation at 50°-60°C for 30 minutes can inactivate common human viruses.[14] For SARS-CoV-2, heating at 56°C for 45 minutes was recommended by the National Health and Health Commission of the People's Republic of China as a standard procedure to inactivate SARS-CoV-2 in clinical samples.[15] A higher temperature is a safer choice for the inactivation of SARS-CoV-2.

We placed the masks inside a steel box to ensure even heating. In some ovens, limited space may require the masks to be placed closely to the heating appliances and cause poor air convection, leading to a higher temperature than intended. A steel box can overcome such obstacles. Notably, this study was aimed to provide recommendations for home use, although medical workers may benefit from the information with caution. The following details should be noted: (1) when masks are overused or used in heavily polluted air, their reuse should be managed with more caution; (2) the oven must be a waterproof incubator or without an exposed heating tube, and (3) a steel box can ensure both uniform heating and safety.

To summarize, dry heat at 60°C and 70°C for 1 hour can ensure the decontamination of surgical face masks and N95 respirator while maintaining their filtering efficiency and shape for up to at least three rounds of dry heat. This practice is suitable for use at home and will dramatically reduce the rapidly increasing need for protective masks globally during a pandemic like COVID-19.

References

1. Wolrd Health Organization. Coronavirus disease 2019 (COVID-19) situation report -68. Available at: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200328-sitrep-68-covid-19.pdf. Accessed March 28, 2020.
2. Rio Carlos del, Malani Preeti N. COVID-19—New insights on a rapidly changing epidemic. *JAMA Network*. 2020. JAMA Published online February 28, 2020 (Reprinted).
3. Nicas M, Nazaroff WW, Hubbard A. Toward understanding the risk of secondary airborne infection: emission of respirable pathogens. *J Occup Environ Hyg*. 2005;2:143–154.
4. Loeb M, Dafoe N, Mahony J, et al. Surgical mask vs N95 respirator for preventing influenza among health care workers: a randomized trial. *JAMA*. 2009;302:1865–1871.
5. Centres for Disease Control and Prevention. Use of cloth face coverings to help slow the spread of COVID-19. Available at: https://www.cdc.gov/coronavirus/2019-ncov/downloads/DIY-cloth-face-covering-instructions.pdf. Accessed June 15, 2020.
6. World Health Organization. Coronavirus disease (COVID-19) advice for the public: when and how to use masks. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/advice-for-public/when-and-how-to-use-masks. Accessed June 15, 2020.
7. Roth B, Mohr H, Enders M, Garten W, Gregersen JP. Isolation of influenza viruses in MDCK 33016PF cells and clearance of contaminating respiratory viruses. *Vaccine*. 2012;30:517–522.
8. Hamamoto I, Takaku H, Tashiro M, Yamamoto N. High yield production of influenza virus in Madin Darby canine kidney (MDCK) cells with stable knockdown of IRF7. *PLoS One*. 2013;8:e59892.
9. OSHA Standard for Respiratory Protection, 29CFR 1910.134. Available at: http://www.chemicalstrategies.org/pdf/training_materials/respiratory_standard.pdf. Accessed June 15, 2020.
10. Dutch National Institute for Public Health and the Environment. Reuse of FFP2 masks. Available at: https://www.rivm.nl/en/documenten/reuse-of-ffp2-masks. Accessed June 15, 2020.
11. Li DF, Cadnum JL, Redmond SN, et al. Steam treatment for rapid decontamination of N95 respirators and medical face masks. *Am J Infect Control*. 2020;48:855–857.
12. Heimbuch BK, Wallace WH, Kinney K, et al. A pandemic influenza preparedness study: use of energetic methods to decontaminate filtering facepiece respirators contaminated with H1N1 aerosols and droplets. *Am J Infect Control*. 2011;39:e1–e9.
13. Vieira FR, Pecchia JA. An exploration into the bacterial community under different pasteurization conditions during substrate preparation (Composting-Phase II) for Agaricus bisporus Cultivation. *Microb Ecol*. 2018;75:318–330.
14. Bertrand I, Schijven JF, Sanchez G, et al. The impact of temperature on the inactivation of enteric viruses in food and water: a review. *J Appl Microbiol*. 2012;112:1059–1074.
15. National Health and Health Commission of the People's Republic of China. Diagnosis and treatment guidelines for 2019 novel coronavirus pneumonia (Draft version 7)[EB/OL] (March 3, 2020). Available at: http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml. Accessed June 15, 2020.