



Disinfection of *Pseudomonas aeruginosa* from N95 respirators with ozone: a pilot study

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ABSTRACT

Introduction Personal protective equipment shortages require the reuse of N95 respirators. We sought the necessary conditions for ozone to disinfect N95 respirators for reuse and the effects of multiple cycles of exposure.

Methods Portions of 3M 1870 N95 respirators were exposed to ozone at 400 ppm with 80% humidity for 2 hours to determine effectiveness of ozone on killing *Pseudomonas aeruginosa*. Entire 3M 1870 N95 respirators were exposed to five cycles of 400 ppm with 80% or higher humidity for 2 hours then evaluated for ozone's effects on airflow resistance, filtration efficiency, strap strength and quantitative fit.

Results Ozone exposure disinfected 3M 1870 N95 respirators heavily inoculated with *P. aeruginosa*. Ozone exposure did not negatively affect the airflow resistance, filtration efficiency, strap strength or fit of the 3M 1870 N95 respirator.

Discussion These results suggest that ozone is a feasible strategy to disinfect N95 respirators for reuse during this and future pandemics.

INTRODUCTION

The COVID-19 crisis has created a shortage of personal protective equipment (PPE), most critically, National Institute for Occupational Safety and Health (NIOSH)-approved N95 filtering facepiece respirators, hereafter referred to as N95 respirators (or N95's) for healthcare personnel (HCP). The Centers for Disease Control (CDC) recognised conditions under which the reuse of N95 respirators may be necessary.^{1 2}

We investigated the feasibility of ozone disinfection of N95 respirators to ensure sufficient supplies for HCP during the situations like COVID-19 pandemic, which has created shortage of PPE. Ozone has been shown to inactivate viruses by acting on the protein structure of a virus capsid or on viral nucleic acids including COVID-19.³⁻⁶ Therefore, it seemed feasible to explore its potential benefit in inactivating COVID-19. For initial investigations, a bacterial surrogate microbe, *Pseudomonas aeruginosa* (PsA), was chosen.

Key messages

- ▶ We aimed to find: (1) conditions necessary for ozone to kill targeted organisms on N95 respirators for disinfection and reuse; and (2) the effect of ozone in similar conditions on the function of N95 respirators after multiple cycles of disinfection.
- ▶ Ozone is a feasible strategy to disinfect N95 respirators.
- ▶ Ozone at 400 ppm with 80% humidity for 2 hours effectively kills *Pseudomonas aeruginosa* on the 3M 1870 N95 respirators. Five cycles of exposure at these conditions do not degrade the filtration efficiency of the 3M 1870 N95 respirators nor does it negatively affect the quantitative fit of the mask.

Ozone can be generated from air, quickly destroyed, naturally degrades to oxygen and is easily accessible through commercial means. We aimed to find: (1) conditions necessary for ozone to kill targeted organisms on N95 respirators for disinfection and reuse; and (2) the effect of ozone in similar conditions on the function of N95 respirators after multiple cycles of disinfection.

METHODS

N95 respirators from 3M (type 1870) were tested. An ozone chamber provided by Ozone Solutions (Hull, Iowa, USA) was used for testing the efficacy of ozone for killing bacteria on N95 respirators. The ozone chamber consists of an airtight chamber, ozone generator using ambient air capable of concentrating to 500 parts per million (ppm), ozone destruction unit and ozone UV analyser.

Four pieces of the N95 respirator approximately 4 cm² were dipped in a culture of PsA overnight. We chose PsA as a representative organism to inoculate our masks because it is a vegetative bacteria that the CDC identifies as more difficult to kill than viruses⁷ and ozone concentrations required to inactivate

vegetative bacteria have been shown to be 4–13 times higher than for viruses.³ Half of the pieces were exposed to ozone: 400 ppm 80% humidity for 2 hours. The remaining half were kept in ambient air approximately 35% humidity for 2 hours. The pieces were dipped in 1 mL phosphate buffer solution and vortexed vigorously to dissociate the maximal amount of bacteria from the surface or interior of the respirator. Serial dilutions were plated on agar plates to enumerate surviving bacteria as colony-forming units per millilitre (CFU/mL). This process was performed twice resulting in four pieces in our ozone-exposed experimental group (n=4) and four pieces in our control group (n=4).

The effects of ozone on filtration efficiency were tested on five whole respirators after exposure to 450 ppm 75%–90% humidity for 2 hours for five cycles with 20 min ambient air breaks. To ensure that there was no residual ozone on the respirators themselves, we used a handheld monitor to measure the ozone level on or near the surface of the respirator. We observed no residual ozone level on or near the respirator. Ozone exposures were performed at Ozone Solutions. Airflow resistance and filter efficiency tests were performed by the CDC (Pittsburgh, Pennsylvania, USA) per protocol in NIOSH N95, 42 CFR Part 84 (Respiratory Protective Devices) (TSI) using 0.26 µm aerosolised sodium chloride under a flow rate of 85 L/min.^{8,9} An additional five whole respirators underwent quantitative fit testing protocol as defined by OSHA 1910.134(f)(7) using a mannequin headform simulating normal and deep breathing. Overall mannequin fit factor (mFFO) scores range from 0 to 200 and are quantitative assessments of fit that can be replicated with other quantitative fit-testing devices. Scoring ranged from 0 to 200, where a score greater than 100 indicates that no change in fit performance is detected. Tensile strength testing of the straps of three respirators was performed to determine per cent change in strap integrity (tensile strength of exposed respirators minus the controls divided by the controls).^{9–11}

Comparisons between ozone-exposed and control groups were made using a two-tailed Student's t-test with threshold for significance defined as $p < 0.05$. No human subjects were involved in this research study.

RESULTS

Exposure to ozone at 400 ppm with 80% humidity for 2 hours effectively killed bacteria on the 3M 1870 N95 respirator, as shown in [table 1](#).

There were no significant changes in filtration efficiency ($p=0.45$) nor filter resistance ($p=0.84$) between ozone-exposed 3M 1870 respirators and controls after exposure to ozone for five cycles with little to no noticeable wear, as shown in [table 2](#). No visible degradation of the 3M 1870 straps was observed, and there were no significant reductions in tensile strength of top (0.2%, $p=0.99$) or bottom strap (5.3%, $p=0.65$).

Quantitative fit testing revealed no loss of fit as a result of ozone exposures. Ozone-exposed respirators scored an average 180 (range=118–200) compared with the control average score 169 (range=121–200).

DISCUSSION

This is the first report demonstrating that an ozone gas application is effective at killing organisms that may contaminate N95 respirators and does not damage or degrade respirator filtration ability.

Ozone achieved high level of disinfection against PsA on N95 respirators, an organism that is more difficult to kill than SARS-CoV-2.⁷ Additionally, our experiments were performed with very high level of contamination (>10 million CFUs/4 cm²). The necessary conditions were 400 ppm ozone for 2 hours with relative humidity 80%. Similar to previous reports, humidity is essential in bacterial killing by ozone and fails to kill pathogens on respirators below 50% relative humidity.¹² Lower concentrations of ozone for shorter periods of time may be effective on viruses based on previous work with influenza virus.^{13,14} Further testing is necessary to determine if such conditions exist.

Ozone did not degrade the function of the respirator filters after five cycles of ozone at 450 ppm at 75%–90% humidity for 2 hours. The filtration portion of N95 respirators consists primarily of polyethylene and polypropylene with which ozone does not easily interact. There was no change in fit detected by quantitative fit testing, and the straps did not experience significant reduction in tensile strength.

Table 1 Log kill efficacy in ozone-exposed respirator pieces compared with the controls

Respirator	Case number	Log CFUs/respirator piece (control group)	Log CFUs/respirator piece (ozone group)	Kill yield log
3M 1870	1.1	8.48	ND	>8
	1.2	9.48	ND	>9
	1.3	7.95	ND	>7
	1.4	8.00	ND	>8

Kill yield log is expressed as the base 10 logarithm of the reduction in organisms. Log 3 = 99.9%, log 6 = 99.9999%, etc. Greater than log 7 kill of *Pseudomonas aeruginosa* bacteria was achieved on heavily inoculated pieces of N95 masks using 400 ppm ozone for 2 hours with relative humidity 80%.

CFU, colony-forming units; ND, not detected.

Table 2 N95 Airflow resistance filtration efficiency and strap integrity testing after exposure to ozone 450 ppm at 75%–90% humidity for 2 hours per cycle

Respirator type	Number of ozone treatment cycles	Mean filtration efficiency±SEM (%) (range)	Mean filter resistance±SEM (mmH ₂ O) (range)	Top strap mean force ±SEM (N) (range)	Bottom strap mean force ±SEM (N) (range)	Overall mannequin fit factor mean ±SEM (mFFO) (range)
3M 1870 N95 Respirator	0 (controls, n=5)	99.42 ±0.43 (99.57–99.87)	8.53 ±0.18 (8.2–8.6)	1.708 ±0.083 (1.581–1.865)	1.753 ±0.028 (1.703–1.799)	172.3 ±10.8 (161–194)
	5 (n=5)	99.83 ±0.04 (99.72–99.93)	8.64 ±0.45 (7.8–10.3)	1.704±0.222 (1.263–1.978)	1.660±0.175 (1.313–1.876)	186.7±3.3 (180–190)

N95 respirators were exposed to ozone at Ozone Solutions and filtration and strap integrity tests were performed at the CDC. Filtration efficiency testing was performed on five respirators. Filtration efficiency tests used the TSI model 8130A per protocol in NIOSH N95, 42 CFR Part 84 (Respiratory Protective Devices) (TSI) using 0.26 µm aerosolised sodium chloride under a flow rate of 85 L/min. Quantitative fit testing was performed on three respirators using a mannequin fit factor (mFFO) with Statist Advanced Headform (Hanson Robotics) following the protocol as defined by OSHA 1910.134(f)(7) where tight-fitting facepieces undergo a pass/fail test to assess for change in fit performance associated with disinfection of respirators. mFFO scores range from 0 to 200 and are quantitative assessments of fit that can be replicated with other quantitative fit-testing devices. Scores greater than 100 are considered to have passed this quantitative fit testing, that is, no change in fit performance is detected. Tests were performed on stationary mannequin headforms simulating normal and deep breathing.^{9–11}

The ozone analyser is critical to this process. The ozone analyser continuously measures the presence of ozone in parts per million before, during and after ozone production. It ensures that a proper concentration is achieved and maintained. After 2 hours of ozone at 400 ppm, the ozone destruct unit was turned on. With the use of the ozone analyser, we ensured that no ozone is left in the chamber. The chamber has internal fans that homogenise the internal atmosphere to ensure no residual ozone lingers within the sealed chamber.

These data suggest that ozone may be an effective and easily accessible method for disinfection of N95 respirators, complementing the methods recently authorised under FDA emergency use authorisations.^{15–21} Future directions will focus on repeating these experiments using additional gram-negative and gram-positive bacteria, phages which are frequently used surrogates for COVID-19, and an airborne non-pathogenic virus. However, further studies are required to directly assess the effects of ozone on SARS-CoV-2.

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Contributors EPM and MDS wrote the manuscript, planned and organised experiments, interpreted the results; SD planned and organised experiments and interpreted the results. BS originated idea of ozone disinfection of N95 respirators and interpreted results; PG and ZG interpreted the results and edited the manuscript; CSCDC provided equipment and supervised experiments. LS planned, organised and performed experiments, interpreted the results and edited the manuscript.

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Competing interests None declared.

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