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ANTIMICROBIAL AGENT TREATED FILTERING FACEPIECE RESPIRATORS FOR INACTIVATION OF AIRBORNE VIRUSES DURING ENVIRONMENTAL CATASTROPHE

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ABSTRACT

Bioterrorism attack within large building using bacterial and viral agents in the form of aerosolized submicron spores may create an unprecedented environmental catastrophe. The filtering facepiece respirator (FFR) can be adopted as the first protection. This study investigated the antimicrobial oxidant's efficacy in diminishing viability of the aerosolized biological agents after passing through the oxidant treated FFR over a period of time. Three FFRs were N95-P100, Triosyn T5000 and Super High Efficiency Particulate Air (HEPA) Medium (SHM). The N95-P100 was the control, whereas, both the T5000 and SHM contained a layer treated with an antimicrobial oxidant. The MS2 bacteriophage agent was aerosolized, passed through the FFRs, and collected in the hot sealed aerosol bags, which were kept for 0-, 1-, 2-, 5-, and 10-minute of residence time before quenching and consuming filter released iodine by a buffer solution (sodium thiosulfate) in all-glass impingers. The viability (plaque forming unit) of the collected microbial agents was quantitated by plaque assays using Single Agar Layer and *Escherichia coli*. The experimental results for all FFRs were in good agreement with the reported literature. The viability of biological agents for SHM FFR exponentially dropped from 100% to 46%, 28%, 12% and 4% over the period of residence time from 0 minute to 1, 2, 5 and 10 minutes, respectively, this can be interpreted that the half-life was about a minute. Reduction of the viability for T5000 and N95-P100 were very low in the order of 1.4% and 3%, respectively, for all four residence times. The SHM FFR provided the strongest lethal dose of disinfectant to the pathogens than that of N95-P100 and T5000, which can be adopted as mass prophylaxis against airborne submicron-deadly viruses during environmental catastrophe.

Keywords: Biocidal filter, MS2 virus, Infectivity inactivation, Removal Efficiency, Iodine Oxidant

INTRODUCTION

An aerosol is a collection of solid or liquid particles suspended in air or a gas (1). Bioaerosols are airborne particles of biological origins including viruses, bacteria, fungi, and all varieties of living matter. Biological weapons are typically bacterial and viral agents (2-4). If a bioterrorism attack took place using bacterial and viral agents, a likely scenario would be aerosolization of spores within a large public building. Spores could be distributed widely through portions of the building using the heating, ventilating, and air conditioning (HVAC) system. Such an attempt to disperse a biological agent in aerosolized form may cause large numbers of casualties and create an unprecedented environmental catastrophe. It is often specified that filters installed in the HVAC system are 95% efficient at removal of 0.3µm particles such as potassium chloride or dioctylphthalate (5,6).

In order to expedite mass prophylaxis in the form of individual or collective protection further, high efficient (>99%) respirators may be used for protection from these agents. A respirator is a personal protective device that is worn on the face, covers at least the nose and mouth, and is used to reduce the wearer's risk of inhaling hazardous airborne particles (including dust particles and infectious viral or bacterial agents), gases, or vapors. It is often used by firefighters, lab scientists, and workers in the manufacturing, construction, steel and cotton plants. The N95 (generic name) respirators are considered as a last-line-of-defense in the occupational hierarchy of controls. It is also reported that the filtering system in an N95 face piece respirator filters out at least 95% of airborne submicron particles upon completion of a worse-case testing scenario with the most-penetrating sized particle, so it is given a

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rating of 95 (7). One of the most essential components in the N95 respirator is the 'electret filter' which carries electrostatic potential. Electret filter media is electrostatically charged microfibers that attract particles due to electrostatic image charge force. Image charge force is the attraction force between uncharged particles and the charged fibers (8,9). If it is possible for an electret filter to achieve 99% filtration efficiency which is excellent, however, a challenge of 10^6/cc biological agents in aerosolized form will penetrate 10,000 agents (1% of 10^6). This is especially significant because many pathogens have a median infectious dose of a hundred particles or fewer. It is thought that for many of the bio-weapon viruses (0.05 µm to 0.3 µm in size), the median infectious dose is <100 plaque-forming units per mL (10). Respirator filters by design in their present form will not be adequate to protect the user from infection when challenged with a hypothetically attainable large load of viruses in the event of biological-weapon attack.

The primary forces that influence a biological aerosol particle be deposited or caught on the respirator filter fibers consist of five electromechanical forces: 1) inertial impaction force, 2) Brownian diffusive force, 3) interceptive (adhesive) force, 4) gravitational force, and 5) electrostatic force (11). However, few thousands of biological agents with mass median aerodynamic diameter of 27 nm which sneaked though the filter can be deadly. It was reported that aerosol of this sizes are small enough to reach the alveolar region of the lungs due to Brownian diffusion and space charge force (carried intrinsic electrostatic charge, if any) (12). Moreover, inhalation of one such single particle can easily attain the minimum infectious dose of virus with enhanced shielding and encasement effects (13).

Previous in vitro studies conducted by various investigators and air purification products manufacturer reported that incorporating poly (styrene-4-[trimethylammonium] methyl triiodide) (PSTI), an oxidative agent to the filter layer provides 2-log increase in viable removal efficiency (VRE) compare to the standard filter layers (14-18). Others' work suggested that the exposure to the treated resin shortened the half-life for deactivation by at least half; quenching experiments in which the aerosols were trapped in thiosulfate medium showed complete loss of effect by the iodine (19). Moreover, trapping in 4% bovine serum albumin caused partial loss of effect of iodine, putting its value for individual protection in question. This addresses utility for collective protection. With these in mind, this study was conceptualized to reduce viability of aerosolized pathogens and decrease the risk that cumulative exposure to personnel breathing a protected air supply will approach the median infectious dose of the pathogens.

The goal of this study was to evaluate PSTI treated and untreated filtering medium for removal and inactivation of aerosolized biological agents. The objectives of the commendable experiments were 1) understand the accelerated attenuation of bioaerosol viability, 2) isolate iodine virus chemistry for controlled periods of time to measure the effect of disinfectant on viability, and 3) determine if disinfection is effective in aerosolized state.

MATERIALS AND METHODS

In order to determine the VRE of three commercially available respirator filtering medium with and without surfaced with PSTI this study developed an experimental system in which viable MS2 aerosols were passed through filtration media and then trapped and held in a reservoir for an arbitrary duration of time before collection in a quenching medium for viable enumeration. The experimental setup consisted of several components. They were 1) A six-jet Collison nebulizer (Model CN25, BGI Inc., Waltham, Massachusetts, USA), 2) A ^{85}Kr charge neutralizer (TSI Inc., Shoreview, Minnesota, USA), 3) Porous tube diluter (Mott Corp., Farmington, Connecticut, USA), 4) Custom fabricated filter holder (Triosyn Corp., Williston, Vermont, USA), 5) Three commercially available Filtering Facepiece Respirators (FFRs): a) N95-P100 Respirator # 8293 (3M Corp., St. Paul, Minnesota, USA), b) Triosyn T5000 and c) Super High Efficiency Particulate Air (HEPA) Medium (SHM) from Triosyn Corp., Williston, Vermont, USA, 6) Heat sealed Food Saver®) air bag (Jarden Consumer Solutions, Rye, New York, USA), 7) Rotameter (Model 034-62G, Cole–Parmer, Vernon Hills, Illinois, USA), 9) Digital flow meter (Model FMA-1601A, Omega Engineering, Stamford, Connecticut, USA), 10) Magnehelic gauge to measure pressure drops across filters, 11) Temp and humidity (TH) meter (Model VWR, Control Company, Friendswood, Texas, USA), 12) AGI-30 impinger (Ace Glass Inc., Vineland, New Jersey, USA)

Test Filters

Samples of the filter discs of 40 mm diameter were cut with a circular punch from three models (N95-P100, T5000 and SHM) of FFR. N95-P100 was the inert control without having PSTI treated layer. Both the T5000 and SHM are fabricated in multiple

layers, of which one is a filtering medium containing PSTI. The T5000 contained a carbon layer downstream to the PSTI layer to prevent iodine off-gassing. The SHM did not have a carbon layer, instead it had a commercial fiberglass HEPA medium impregnated with fused PSTI dust.

Test Biological Agents

MS2 bacteriophage (ATCC® 15597-B1™) was selected as representative biological agent and used to generate bioaerosol i.e., virus aerosol. There were several reasons for selecting MS2 as model virus. It is a non-enveloped, icosahedron-shaped, single-stranded RNA with single-capsid size of 27.5 nm, and it perfectly infects and replicates itself inside *Escherichia coli* (20). Like other RNA enteroviruses which are pathogenic to humans, MS2 does not have lipid component surrounding the protein coat and shows similar resistance to antimicrobial agents (19,21). Freeze-dried MS2 was suspended with filtered sterile deionized (DI) water to a concentration of 10^{11} PFU/mL as the virus stock suspension and stored at 4°C. The appropriateness of using ultrasonic nebulization technique to generate virus aerosols of MS2 and the required operating conditions to study their viability have been demonstrated in several studies (14,16). We adopted this methodology.

Experimental Setup And Procedure

A schematic of the experimental setup is presented in Figure 1. Dry and filtered compressed air of 11.3 Lpm (at a pressure of 20 kPa) was applied to the six-jet Collison nebulizer and was used to generate aerosols containing biological agents (MS2 coli phage), and passed through an electrical conductive tubing connector. Conductive

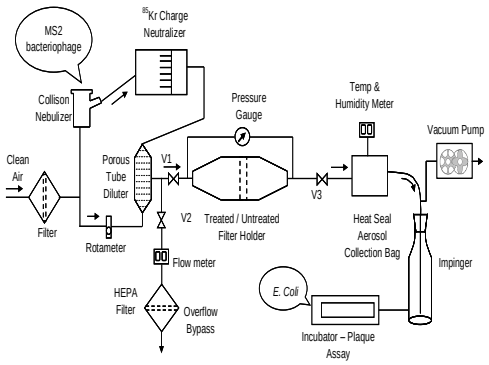

Fig 1. Schematic of the experimental setup to determine the antimicrobial oxidant's efficacy in diminishing viability of the aerosolized bacterial agents upon passing through the treated/untreated respirator filters over a period of time.

connectors were used for all connections because it has low potential for attracting aerosol particles. The ^{85}Kr Charge Neutralizer was used to relieve intrinsic electrostatic charges of aerosol. The custom fabricated filter holder held a 40 mm disc of filter medium. The virus concentration in the nebulizer was $25x10^6$ – $50x10^6$ PFU/mL and was prepared by diluting 3 ml of virus stock suspension in 27 ml sterile de-ionized water. Using a nebulizer solution of this concentration previous *in vitro* studies suggested that a six-jet Collison nebulizer with an air flow rate of 11.3 Lpm can give acceptable uniform loading of viruses in the order of ~10^3 PFU/Liter of air (15). In order to examine the collection efficiency of the filter medium of a FFR, the National Institute for Occupational Safety and Health (NIOSH) recommends the face velocity of 14.2 cm/s through the filter medium (22).

To increase the aerosol flow to the NIOSH recommended face velocity of 14.2 cm/s through the filter medium, HEPA-filtered dilution air was added through a "*T*" fitting at the base of the rotameter which was inserted between the charge neutralizer and the filter holder. This face velocity is typically used by the respirator manufacturers as well, which corresponds to the NIOSH certification of testing of 100 cm^2 media against the 85 Lpm flow rate. We adopted this methodology. The filter holder was connected through threaded fittings at each end, which allows a convenient exchange of holders and challenges to several samples during a nebulizer run. Pressure drop at each filter was monitored with Magnehelic gauge measuring 0-1500 Pa, and recorded at each time while aerosol was allowed to pass the filter.

The relative humidity (RH) of loaded aerosol bags was attained ~90% purposely by adjusting inlet air flow to the nebulizer and mixing air in the diluter. Previous study on MS2 sensitivity to RH showed that at low RH (<50%) the spontaneous rate of loss of viability was fast enough that no PFU remained after the first 15-minute of residence time in aerosol state, whereas, raising the RH to 90% extended the airborne lifetime of MS2 and countable enough to undertake this study (23).

A "*Y*" connector was placed before the filter holder, from which an open leg controlled by valve V2 leaded to feed into a digital flow meter, and then into a HEPA filter which was vented to the hood as a guard against over-pressurization and burdening of filters with viruses while aerosol was not collecting in the heat-sealed aerosol bag i.e., valves V1 and V3 were closed. The second leg from the *Y* connector was controlled by a ball valve V1. When this valve was open the aerosol was allowed

to pass through the filter (inside filter holder). The exiting aerosol from the filter holder was then directly entered into the heat-sealed aerosol collection bag. By opening and closing ball valve V3 the timing to fill the aerosol bag could be controlled.

The heat sealed unfilled bag's dimension was 58.9 cm x 25.9 cm with a filling capacity of 7.53 L and the relative humidity was measured 90% upon loading with aerosol. Each bag was tested for leaks before starting the experiment. By closing valve V1, the system was initially allowed to run for 10 minutes to bring it to the steady state, whereupon the ball valve V2 was closed and valves V1 and V3 were opened to divert the filtered aerosol into the eat-sealed bags for 40 seconds. After loading, the bag was sealed by closing the ball valve (not shown), disconnected from the rig and carried to the collection room. Each sealed bag was allowed to incubate for a control period (i.e., 0-, 1-, 2-, 5-, 10-min) according to the experimental protocol before quenching. Thereafter, the entire content of the bag was drawn by a vacuum pump into an AGI-30 impinger containing 20 mL of 1X PBS and 1% sodium thiosulfate. These steps were repeated alternatively six times. The filter holder was then replaced with a filter holder containing no filtration medium and three bags were filled and collected into the impinger by the same procedures. After the experiment, the impinger solutions were serially diluted and plated in triplicate, and incubated overnight at 37^{o}C. Plaques or colonies were counted the next day. Thus, the viable MS2 bacteriophages in the impingers were quantified using standard single-layer plaque assays (24).

Biological Agent Removal Efficiency Studies

MS2 aerosols were delivered through a 40 mm disc cut from a N95-P100, T5000 and SHM FFRs under the experimental conditions described above. Each filter was tested for one control (0 min) and four residence (incubation) times (RTs) such as 1-, 2-, 5-, and 10-min. In its initial stage while conceptualizing this study we have found that VRE is almost 100% after 15 min of RT. Therefore, this work was designed to examine decay studies for 1-, 2-, 5-, and 10-min. Table 1 shows the matrix of the number of aerosol bags tested according to the experimental plan. The matrix of the Table 1 shows total 12 (4 RT x 3 filters) experimental scenarios. Each scenario is an independent experiment. Before and after the experiment, the nebulizer, filter disc holder and MS2 solution beakers were decontaminated by wiping with isopropyl alcohol and placing the whole experiment-set in the decontamination chamber for 24 hrs.

A total of 108 bags (9 bags per set of conditions x 3 FFR mediums x 4 time delays) were filled with aerosols during the entire investigation, of which each bag was incubated according to the residence time (0-/1-/2-/5-/10-min) prior to being emptied into separate AGI-30 impingers for plating and viable enumeration. Each day, the filter element was removed from the test rig upon filling six bags, and three more bags (filled without filter) were collected and immediately sampled into an impinger for plating to quantify the challenge.

In calculating biological agents concentration the dilution factor was accounted, which depends on the number of transfers of the impinger

Table 1: Matrix of the number of aerosol bags tested according to the experimental plan

Residence Time (min)	N95-P100 FFR Untreated	T5000 Treated	SHM Treated
1	Control n = 3, 0 min Test n = 3, 1 min No Filter n = 3	Control n = 3, 0 min Test n = 3, 1 min No Filter n = 3	Control n = 3, 0 min Test n = 3, 1 min No Filter n = 3
2	Control n = 3, 0 min Test n = 3, 2 min No Filter n = 3	Control n = 3, 0 min Test n = 3, 2 min No Filter n = 3	Control n = 3, 0 min Test n = 3, 2 min No Filter n = 3
5	Control n = 3, 0 min Test n = 3, 5 min No Filter n = 3	Control n = 3, 0 min Test n = 3, 5 min No Filter n = 3	Control n = 3, 0 min Test n = 3, 5 min No Filter n = 3
10	Control n = 3, 0 min Test n = 3, 10 min No Filter n = 3	Control n = 3, 0 min Test n = 3, 10 min No Filter n = 3	Control n = 3, 0 min Test n = 3, 10 min No Filter n = 3

solution. The viral concentration in the collected impinger solution, C_v (PFU/mL), was determined as

$$C_v = \frac{PFU}{10^{-n} \times V} \quad (1)$$

where *PFU* is the number of plaque forming units, *V* is the volume of diluted solution and *n* is the dilution factor. The final mean viral concentration was calculated by averaging all values in each dilution.

The removal efficiency can be expressed as VRE, which can be determined as

$$VRE(\%) = \left(1 - \frac{N_{RT}}{N_0}\right) \times 100 \quad (2)$$

where N_{RT} is the number of virus PFU at RT min residence time, and N_0 is the number of the virus PFU at 0 min residence time. The VRE depends on the infectivity of the I_2, oxidizing agent that was carried by each bioaerosol particle while passed though the filter.

plaque assays of the viruses collected in the impingers. Table 2 shows the MS2 bacteriophage concentration and VRE of iodine oxidant treated and untreated filters at various control periods of residence time.

The Cvs for uniform deposition of aerosolized viral particles onto substrates for different tested filters were calculated by analyzing the infectivity of viruses extracted from the loaded filter. At the given experimental environment (RH ≈ 90%, Temp 23°C, volumetric flow rate 11.3 Lpm, and face velocity 14.2 cm/s) viability of MS2 declined with respect to the residence time. As a result, we have observed increase in VRE for all three filters. Operational variation such as new MS2 stock solution for each scenario may contribute to the differences in Cvs for the same filter but different RT. Therefore, the same stock solution was used to generate aerosolized viral loading for 0-min control RT and RT of interest. The average penetrations of the MS2 through each of the three

Table 2: MS2 bacteriophage concentration and removal efficiency of iodine oxidants treated and untreated filters at various control periods of residence time

RT (min)	N95-P100		T5000		SHM	
	Cv (PFU/mL)	VRE (%)	Cv (PFU/mL)	VRE (%)	Cv (PFU/mL)	VRE (%)
1	299 ± 24	14 ± 2	574 ± 39	3 ± 1	420 ± 96	54 ± 10
2	50 ± 15	19 ± 3	46 ± 6	5 ± 2	284 ± 22	72 ± 9
5	158 ± 24	25 ± 7	72 ± 5	7 ± 3	133 ± 39	88 ± 6
10	228 ± 24	32 ± 9	59 ± 8	11 ± 5	53 ± 34	96 ± 4

RT: residence time in minutes (holding time of aerosol filled bags before quenching), N95-P100: control filter without treatment, T5000: Triosyn respirator filter with iodine oxidants treated, SHM: super high efficiency particulate air medium filter with iodine oxidants treated, Cv: MS2 bacteriophage concentration in PFU/mL (mean ± Std. Dev.), PFU: plaque forming unit, VRE: viral removal efficiency in percent (mean ± Std. Dev.), Note: bags were alternately evaluated for the control and test population

RESULTS AND DISCUSSION

This investigation was designed to search for and quantify antimicrobial activity transferred to microbes penetrating air filter fibers containing PSTI during incubation lasting for minutes before quenching all of the iodine chemistry and viable enumeration. The pressure drops of the N95-P100, T5000 and SHM filters were recorded 174 ± 17, 585 ± 32, and 981 ± 10 Pa respectively. The possible attenuation mechanism of biological agent (MS2 viruses) viability were considered: (i) the inactivation of viruses downstream of the treated filter by reaction with iodine oxidants (I_2) released from the filter, and (ii) transfer of iodine oxidants during close contact of virus aerosol while passing through the treated filter. The VRE of the untreated and treated filters was calculated by

tested filters were 347-, 609-, and 977-PFU/mL for N95-P100, T5000 and SHM, respectively.

The performance in shielding MS2 penetration is not a surprise because each of these filters possesses above 99.970% filtration efficiency as specified by the manufacturers, and thus penetration would be expected. In detail, given the challenge loads of ~33×10^6 PFU the penetrations were expected to be greater than what were observed. However, the test measures only viable MS2s and there may be mechanical shear forces that were killing some of the viruses as they were penetrating the filters. Therefore, it is clear that viable viral particles penetrated the filter in amount well above the bio-weapon counts (see Introduction).

One may observe that the VREs did not sharply increase with the increase of residence time for the N95-P100 and T5000 filters. In contrast, 50% viral removal took place after the first minute and another 50% of the remaining removed by the next minute. Additionally, generation of aerosols at each scenario started with a new MS2 virus stock solution. Although, the challenged virus counts (PFU/mL) for each scenario was comparable with that of the other scenarios in the Table-1, they were not exactly the same. To accommodate this issue for each scenario, six bags of aerosols were filled alternatively (one for 0 min control RT and the next for the tested RT). This methodology allowed us to challenge a particular type of filter with consistent (comparing apple-to-apple) counts of virus loading. The SHM filter, which was not backed by a carbon layer, caused viable counts of the virus to decay exponentially (0% - 96%), with a half life of about a minute. Data were statistically significant ($P < .05$) from both the T5000 and N95-P100 control data.

The water–iodine disinfection chemistry was reported in another study with aerosols of MS2 coli phage by passing through P100 (control) and iodinated HEPA media (8). It showed that disinfection of viruses can be achieved but insignificant in clinical point-of-view to protect individuals but it can minimize the spreading of infections in closed buildings. The biochemical mechanism by which the PSTI is reducing viral particles is thought to take place by the viral particles coming in contact with the iodine. As organisms pass through the filter, iodine is also selectively released and killed the organisms. Besides, viral particles were about 27.5 nm in sizes which not only caused low flow-ability intrinsically but also experienced high cohesion due to adhesive forces arising from surface tension of absorbed liquid films on organic virus molecules (1).

The present experiment suggests that oxidant treatment of respirator filters enhanced collective protection from pathogenic viruses if they are challenged in a finely aerosolized form. Here it is shown that iodine from the SHM is effective against the aerosol on a time scale in minutes, but at least two factors confound the interpretation of the result. 1) Rengasamy et al. (2010) measured I_2 concentrations that were toxic to MS2 in water downstream of PSTI-containing media absent the backing carbon layer used in the marketed product [23]. The quenching medium (sodium thiosulfate) used in this work eliminates the possibility of interference from toxicity of free (and bound) I_2 in the impinger. Therefore, the sources of I_2 causing toxicity during incubation of the aerosol were from oxidant treated layer. 2) The same rationalization proposed an uncharacterized distance-dependent factor for probability of capture of I_2 during passage by any fiber.

The distance between fibers in the T5000 medium, functionally an N99 electret is much greater than in the SHM, which is a fiberglass HEPA medium, so the obvious extension is that capture of I_2 in the narrower spaces of the SHM should be much more efficient than in the wide open spaces of the electret and if the attenuation observed for the T5000 is genuine, the set of results would be precisely consistent with the mechanism proposed. However, one control experiment remains to be performed: a small I_2 background is produced by passage of air through the filter, and passage of charged aerosols by the iodinated fiber could be expected to release additional I_2 into the air if some of the I_2 molecules were not captured. To test these possibilities it will be necessary to measure the iodine concentration in air after passage of nothing and of an MS2 bioaerosol in the same volume of air, and to deliver an MS2 aerosol into air containing I_2 vapor at the same final concentration measured in the bioaerosol penetration control. The difference in attenuation rates between this control and the values in Figure 2 will be the effect of the direct attachment mechanism. Although, the present work demonstrated disinfection of one type of biological agent (MS2 bacteriophage) by contact-acquired iodine oxidants, general characteristics applicable to other viral aerosols can be induced from our findings.

The firm conclusion we can draw is that iodine chemistry (antimicrobial agent) kills airborne viruses in contact. The viability of the viruses diminishes aerosol state at high humidity over time but passing through iodine-treated filters accelerated the rate of de-vitalization by an order of magnitude. However it is a slow process. Furthermore, control experiments are needed to isolate the contribution of free iodine vapor to this acceleration. The SHM FFR provides the strongest dose of disinfectant to the pathogens than the N95-P100 and T5000 FFRs, which can be adopted as mass prophylaxis against airborne submicron and deadly viruses during environmental catastrophe at large buildings with high occupancy.

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