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AEROSOL-PHASE ACTIVITY OF IODINE CAPTURED FROM A TRIIODIDE RESIN FILTER ON FINE PARTICLES CONTAINING AN INFECTIOUS VIRUS (POSTPRINT)

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14. ABSTRACT

The triiodide form of an anion-exchange resin—an effective antimicrobial in water—is claimer to attenuatei nfectivity of airborne microorganisms. However, attempts to evaluate the effect of passage through a filter medium containing the triiodide resin were impeded by reactions of iodine with proteins during collection and with water during analysis. Aerosols of MS2 coli phage were passed through control P100 and iodinated HEPA media, collected in plastic bags, incubated for 0–10 min, collected in an impinger containing thiosulfate to consume unreacted iodine, plated and enumerated. Comparison of viable counts demonstrated antimicrobial activity with an apparent half-life in tens of seconds. Antimicrobial effectiveness increased with relative humidity. Absorption of iodine by the bags eliminated reaction with free iodine as a contributing mechanism. The iodinated medium appears to have possible use in collective, but likely not individual, protection.

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Antimicrobial, biological aerosols, coli phage, filter, infection, iodine, triiodide

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ORIGINAL ARTICLE

Aerosol-phase activity of iodine captured from a triiodide resin filter on fine particles containing an infectious virus

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Keywords
antimicrobials, contact release mechanism, iodine, MS2 coli phage, respiratory protection, viruses.

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Abstract

Aims: To avoid interference by water iodine disinfection chemistry and measure directly the effect of iodine, captured from a triiodide complex bound to a filter medium, on viability of penetrating viral particles.
Methods and Results: Aerosols of MS2 coli phage were passed through control P100 or iodinated High Efficiency Particulate Air media, collected in plastic bags, incubated for 0 10 min, collected in an impinger containing thiosulphate to consume all unreacted iodine, plated and enumerated. Comparison of viable counts demonstrated antimicrobial activity with an apparent half life for devitalization in tens of seconds; rate of kill decreased at low humidity and free iodine was captured by the bags.
Conclusions: The results support the mechanism of near contact capture earlier proposed; however, the disinfection chemistry in the aerosol phase is very slow on the time scale of inhalation.
Significance and Impact of the Study: This study shows that disinfection by filter bound iodine in the aerosol phase is too slow to be clinically significant in individual respiratory protection, but that it might be of benefit to limit airborne transmission of infections in enclosed areas.

Introduction

Recurring reminders of the risk of respiratory infection by airborne pathogenic microbes include malicious events (Khan and Ashford 2001) such as the anthrax letters (National Research Council 2011) and spontaneous viral outbreaks including SARS (CDC 2005) and influenza (Bresee and Hayden 2013). The technology of choice for respiratory protection has been and remains particle filters (Hinds 1999), both in ventilation systems and in individual respiratory protective devices, including the N95 filtering facepiece respirator (FFR). Improvements in filtration have been realized with the introduction of electret and nanofibre media (Brown 1993). More recently, research has been directed towards enhancing protection against the airborne infections by the integration of antimicrobials such as silver (Foss Manufacturing Company, Inc. 2004; Miaśkiewicz Peska and Łebkowska 2011) and copper (Copper Development Association 2007; Borkow *et al.* 2010) into filtration media. These additives are effective against micro organisms that are captured by the filtration medium, but exert little or no effect upon pathogens that penetrate the filter. Developing a filtration medium with downstream antimicrobial activity for penetrating pathogens would enhance the protection against respiratory infection afforded by personal respiratory devices and filtering ventilation systems.

The efficiency of polystyrene *co* polystyrene 4 (trimethylammonium)methyl triiodide ion exchange resin (PSTI) as a broad spectrum disinfectant in water was documented half a century ago by Taylor *et al.* (1970) The same resin was later patented (Messier 2000) as a reactive component of air filtration media, which were designed to enhance respiratory protection against

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pathogenic aerosols. The antimicrobial action of PSTI can be attributed to the reaction of iodine with certain amino acids resulting in disruption of microbial proteins. Ratne sar Shumate *et al.* (2008) proposed that in aerosols this interaction occurs following near contact transfer of iodine to microbial particles as they penetrate through an air filter. However, verification of PSTI's antimicrobial mechanism and evaluation of clinical significance will require definitive measurements of antimicrobial activity. Accurate antimicrobial measurements are hindered by interfering processes, including competitive uptake of iodine by various proteins (Muus *et al.* 1941; Lee *et al.* 2009; Eninger *et al.*, 2008), reversibility of iodine attach ment (Lee *et al.* 2009) and the unavoidable use of water based analytical media, which activate an efficient kill mechanism (Hatch *et al.* 1980) that obscures effects in the aerosol phase.

Two important factors must be taken into consider ation to understand the activity of iodine in an aerosol environment: the behaviour of iodine acquired in the aer osol state, and the interaction of iodine with endogenous proteins. The usual aqueous or agar media culture meth ods for viability determinations obscure the earlier activ ity of iodine during aerosol interactions. Collecting iodine exposed aerosol samples in impingers containing sodium thiosulphate has proven useful at quenching available iodine, thereby eliminating further iodine effects during culture (Lee *et al.* 2009; Lore *et al.* 2011). From a practical standpoint, the proclivity of iodine to interact with proteins implies competition for iodine between microbial proteins and endogenous proteins of the respi ratory mucosa in humans (Lee *et al.* 2009). Thus, any antimicrobial activity imparted during the filtration pro cess must be expected to experience attenuation at the point of aerosol contact with the mucosal surface.

The effect of environmental conditions on viability and stability of bioaerosols is known to be profound (Sobsey and Meschke 2003; Tang 2009). The composition of neb ulization fluid, relative humidity (RH) and temperature are three important aspects known to prolong or truncate the infectivity of viral aerosols. MS2 coli phage an imperfect (Appert *et al.* 2014) but convenient surrogate organism widely used to represent viral aerosols has been used in PSTI aerosol studies, but these did not dis criminate time dependent effects of PSTI upon viral par ticles under various environmental conditions. Therefore, development of new methods is necessary to approach the question of infection prevention from the use of a PSTI treated filtration medium.

The study herein describes an aerosol method to quantify the antimicrobial effect of iodine acquired from PSTI by free infectious bioaerosols following passage through a filtration medium. Such an accurate determination of the effect of PSTI heralds a tool that can guide discovery and refinement of new applications in aerosol filtration and prevention of infection. This paper details experiments designed to take into account the effects of free and captured iodine in the aerosol state, and to follow those effects over time under varied environmental conditions by utilizing an aerosol incubation system downstream of the filter medium, fol lowed by reduction in residual iodine concurrent with col lection of the aerosol for quantification of infectious virus. Accurate quantification of the clinical effect of captured iodine on respiratory transmissibility of infection, including possible contribution by sublethal effects of iodine exposure on infectivity, can be achieved only by testing in an animal model (Stone *et al.* 2012; McDonald *et al.* 2012).

Materials and methods

MS2 bacteriophage

MS2 bacteriophage (ATCC 15597 B1) was propagated in *Escherichia coli* (ATCC 15597) using standard EPA proto cols (USEPA, 1984). Phage titres were determined using a standard, single layer, plaque assay (USEPA, 1984). For aerosolization studies, MS2 bacteriophage was diluted to a concentration of 10^{10} plaque forming units (PFU) ml^{-1} into sterile deionized water.

Filtration media

Two types of filtration media were used for this study: (i) Triosyn® Super High Efficiency Particulate Air (HEPA) medium incorporating poly(styrene *co* styrene 4 [trime thylammonium]methyl triiodide) (PSTI) (ds_{50}, provided by Triosyn Corp., Williston, VT), an antimicrobial agent; and (ii) a 3M® 8293 P100 FFR (8293) (3M Corp., St. Paul, MN), a nonantimicrobial filter that exhibits mechanical properties similar to those of the PSTI HEPA medium. Samples of each were cut into 46 mm diameter coupons to be used within the aerosol system.

Experimental setup

A schematic of the aerosol system is presented in Fig. 1. In Fig. 1(a), HEPA filtered, dry, compressed air was supplied to a six jet Collison nebulizer (Model CN25; BGI Inc., Waltham, MA), operating at ~17·5 psi and containing 30 ml of MS2 coli phage diluted to a concentration of ~10^{10} PFU ml^{-1} in sterile water. The nebulized aerosol passed through optional diffusion dryer(s) (Model 250; ATI, Baltimore, MD), which allowed for adjustment of the RH within the system, then through a ^{85}Kr charge neutral izer (TSI, Shoreview, MN), which restored the intrinsic electrostatic charge distribution of the particles. From the

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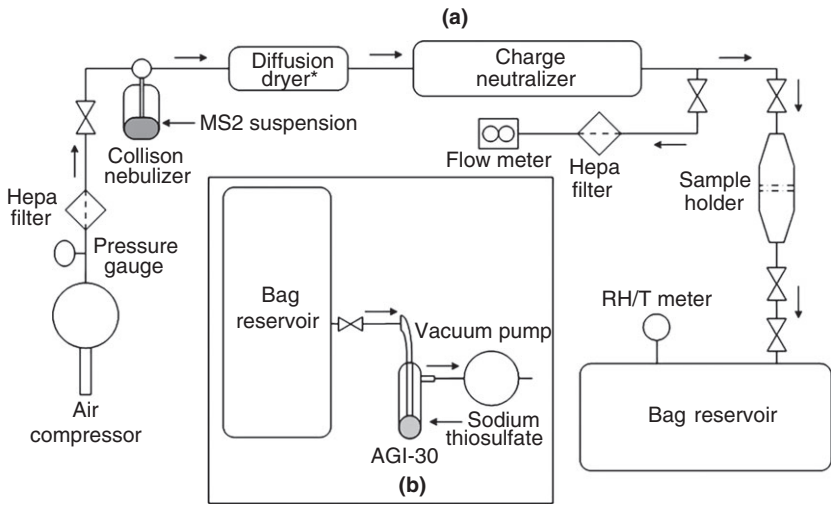

Figure 1 (a) Aerosol capture (b) Aerosol evacuation. (*), 1 3 diffusion dryers were connected in series to adjust RH.

charge neutralizer, the aerosol passed through a 12·7 mm *Y* fitting (Swagelok, Solon, OH) and was directed by one of a pair of ball valves to either a HEPA filtered, overflow bypass whose flow rate was measured, or through a custom fabricated holder containing a 40 mm disc of exposed filter medium (8293, or PSTI HEPA). All components downstream of the Collison nebulizer were connected using 7·9 mm ID, 12·7 mm OD conductive electrical tubing (TSI) to minimize particle attraction. Downstream of the filter holder, conductive tubing directed the aerosol into a heat sealed plastic bag (Food Saver®; Jarden Consumer Solutions, Rye, NY), measuring 58·9 cm × 25·4 cm. Two ball valves allowed disconnection of the bag from the aerosol generation system, creating a sealed reservoir in which the aerosol sample incubated.

Prior to each experiment (Table 1; $n = 18$ experiments), six bags were individually inflated and leak checked using Snoop™ (Swagelok, Highland Heights, OH). For sampling, each bag was filled for 40 s at 11·3 l min^{-1} (LPM) of the MS2 coli phage aerosol and then disconnected from the aerosol generation system, creating an air sample of 7·5 l. Each incubated sample bag was paired with a nominal $t = 0$ control bag that was filled immediately prior to the mated sample, providing a baseline MS2 concentration for comparison. For each experiment, seven aerosol samples were taken: three incubated bags, three baseline bags (one sampled directly before each incubated bag) and an unfiltered sample to assess the concentration of the aerosol challenge. Once disconnected, samples were allowed to incubate at room temperature for various times (Table 1). At the end of the incubation period each air sample was evacuated at ~12·5 LPM through conductive tubing into an All Glass Impinger (AGI 30; Ace Glass, Vineland, NJ), containing 20 ml of 1× phosphate buffered saline plus 1% sodium thiosulphate (Fig. 1b). After collection, the impinger solutions were serially diluted, plated in triplicate and incubated overnight at 37°C. Plaques were then enumerated using a Synbiosis ProtoCol SR colony counter (Cambridge, UK).

Table 1 Experimental layout

	Incubation times (min)		
	86% < RH < 91%	45% < RH < 58%	27% < RH < 35%
Control	0	0	0
8293	1	1	1
	10	10	10
PSTI HEPA	1	1	1
	2	2	2
	5	5	5
	10	10	10

RH, relative humidity; PSTI HEPA, polystyrene *co* polystyrene 4 (trimethylammonium)methyl triiodide ion exchange resin High Efficiency Particulate Air.

Iodine control experiments

Triplicate aerosol samples with and without MS2 in the nebulization fluid were collected using the same experimental setup to quantify available iodine from the PSTI HEPA filter. The only differences were downstream of the coupon holder. No bag was attached; that line was attached instead to a midget impinger (SKI, Eighty Four, PA) filled with 3 mm glass beads and 10 ml of $NaHCO_3$/Na_2CO_3 (1·5 mmol l^{-1} $NaHCO_3$, 1·5 mmol l^{-1} Na_2CO_3) in sterile water, through which the HEPA filtered vacuum pump drew 0·5 LPM of the stream; the remainder of the stream was diverted through the second leg of the

Y junction into a HEPA filter, which served as an over flow. Air through the impinger was metered with a bead flowmeter (Omega Engineering, Stamford, CT) and a sec ond bead flowmeter monitored flow in the overflow stream to maintain the overall flow at 11·3 LPM.

Positive control samples for iodine were collected using the initial experimental setup, modified by addition of a self sealing injection port attached to each bag with an adhesive. Approximately 100 mg of iodine crystals were stored at 35°C in an Erlenmeyer flask sealed with a rub ber stopper for 3 days to reach an equilibrium concentra tion of iodine vapour in the headspace. As each positive control sample was collected in a bag, 0·75 ml was drawn from the headspace into a 1 ml syringe and needle and immediately injected through the port into the sample bags. Bags containing MS2 bioaerosol were incubated for 10 min before evacuation as above at 0·5 LPM through a midget impinger charged with $NaHCO_3/Na_2CO_3$ (Gooch and Valker 1905; OSHA 1994). The negative control was bags filled with clean air and processed similarly immedi ately after filling. Iodine samples were analysed on a Dio nex AS 15 column in an isocratic sodium hydroxide eluent and a Dionex DX 500 high pressure liquid chro matography system with a GP 40 gradient pump; the ED 50 electrochemical detector used a silver working electrode (Dionex Corporation, Sunnyvale, CA) (Meli chercik *et al.* 2006). To prevent possible interference by virus, impinger samples containing MS2 were filtered through a 30 kDa regenerated cellulose membrane before ion chromatographic analysis.

Data analysis

To determine the reduction in viability, the ratio of the MS2 concentrations in the incubated and nonincubated samples was calculated as shown in Eqn (1). A two tailed, *t* test was used to compare the 8293 and PSTI HEPA data for each humidity level. A one way ANOVA with a Bonfer roni post test was used to compare the data obtained from different RH levels for each filtration medium.

$$\text{Fraction of viable MS2 remaining, } V_R = C_i/C_0 \qquad (1)$$

where, C_i = concentration of viable MS2 from incubated sample, PFU ml^{-1}; C_0 = concentration of viable MS2 from nonincubated sample, PFU ml^{-1}.

Results

The average concentration of the MS2 aerosol challenge was $1{\cdot}20 \pm 0{\cdot}93 \times 10^8$ PFU/L_{air}. The 8293 filter demonstrated an average filtration efficiency of 99·999 ± 0·001%, while the average filtration efficiency of the PSTI HEPA medium was 99·975 ± 0·029%. Figure 2 displays the data obtained from each time point (1 and 10 min after filling) at each of three RH levels (27% < low RH < 35% RH; 45% < mid RH < 58% RH; 86% < high RH < 91% RH) for the 8293 filter medium. A one way ANOVA with a Bonferroni post test indicates that the data from the high humidity level for the 8293 are significantly different from the mid and low humidity levels ($P = 0{\cdot}01$) and indicate a half life for loss of viability of roughly half an hour.

Figure 3 illustrates the data obtained for each time point (1, 2, 5, 10 min of incubation after filling) at all three RH levels for the PSTI HEPA medium. A one way ANOVA with a Bonferroni post test of the PSTI HEPA media indicates a statistically significant difference between the low RH level and both the high and mid lev els ($P = 0{\cdot}002$). A two tailed, unpaired *t* test comparing the 8293 and PSTI HEPA media at high, mid and low RH levels showed a statistically significant difference for each condition ($P = 0{\cdot}02$, 0·02 and 0·04 respectively).

No evidence of residual free iodine was detected in any of the iodine control experiments.

Discussion

It was assumed that the untreated medium exerts no effect on penetrating particles. Lee *et al.* (2009) showed that capture of iodine exposed bioaerosols into an impin ger containing thiosulphate immediately terminates the activity of captured iodine, which here allows isolated observation of the effect of iodine on the aerosol particle. Filling of the bag avoided the complication of displacing air from a fixed volume, but consumed 40 s, and empty ing it into the impinger another 37 s; allowing 13 s han dling time to disconnect from the charging system,

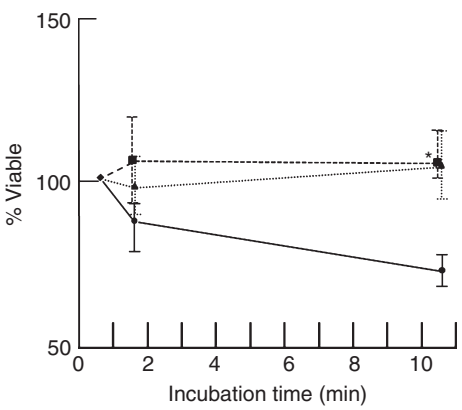

Figure 2 Percent of viable MS2 remaining after incubation at three relative humidity (RH) levels using the 8293 filter medium (*n* 3; **n* 6); the diamond represents the nominal *t* 0 value, to which the subsequent time points are scaled. The circle, square and triangle indicate measurements at high (86% < RH < 91%), medium (45% < RH < 58%) and low RH (27% < RH < 35%) respectively.

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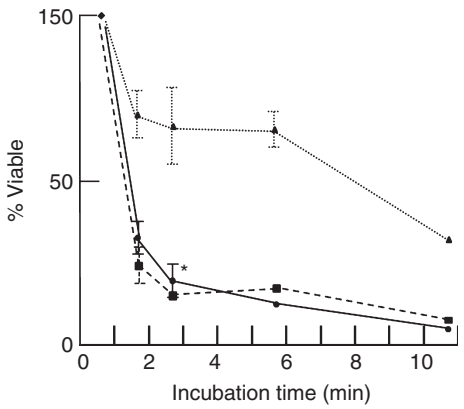

Figure 3 Percent of viable MS2 remaining after incubation at three relative humidity (RH) levels using the PSTI HEPA filter medium (*n* 3; **n* 6); the diamond represents the nominal *t* 0 value, to which the subsequent time points are scaled. The circle, square and triangle indicate measurements at high (86% < RH < 91%), medium (45% < RH < 58%) and low RH (27% < RH < 35%) respectively. PSTI HEPA, polystyrene *co* polystyrene 4 (trimethylammonium)methyl triiodide ion exchange resin High Efficiency Particulate Air.

connect to the impinger and open the valves (a total of 90 s), and assuming linear rates of filling and emptying, we estimate that the aerosol in the nominal $t = 0$ control had functionally been incubated for an average of 45 s. This is recognized in Figs 2 and 3, in which the other time points are shifted by the same amount from the point at which filling was initiated.

Disappearance of added free iodine from all of the iodine control experiments (which were conducted after the bioaerosol data had been gathered) indicates that the vinyl polymer of sample bags rapidly (<10 s) and effi ciently scavenged free iodine. Qualitatively, the observa tion of accelerated loss of viability in the PSTI exposed bioaerosol thus indicates that, as interaction with free iodine vapour ends in a few seconds or less, the near contact transfer proposed by Ratnesar Shumate *et al.* (2008) is indeed a mechanism acting in these systems. Quantitatively, it suggests that the half life of ~1 min reported for loss of viability (Ali *et al.* 2011) and the val ues of the same order shown in Fig. 3 may be slightly longer than the actual value in an inert environment, but that they are of the correct order of magnitude.

Loss of particle bound iodine is possible during wall collisions; however, the duration of the trends in Fig. 3 shows that if this mode of loss competes it is slow, and suggests that the particles may outcompete the wall poly mer to retain captured iodine.

Figure 3 shows that rate of kill increases as RH i.e. availability of water increases. The interpretation most consistent with precedents is that iodine is captured from PSTI as proposed by Ratnesar Shumate *et al.* (2008), and that killing occurs by the same water mediated mecha nism that Hatch *et al.* (1980) proposed in solution; how ever, high humidity might also facilitate transfer of iodine to or capture of iodine vapour by micro organ isms passing through the filter. The time scale of Fig. 3 places the half life for loss of viability in tens of seconds, which is long on the time scale of particle travel from breathing zone to tissue during respiration. As Lee *et al.* (2009) demonstrated concentration dependent intercep tion of bound iodine by a dissolved protein at physiologi cal concentration, clinical benefit from use of such materials in a respirator is, as suggested by Ali *et al.* (2011), likely to be small if any.

In extreme contrast to respiration (individual protection), the usual time scale for air changes in collective protection and heating, ventilation and air conditioning (HVAC) envi ronments is 10 min. As (i) this is at least 10 half lives for loss of viability after passage through a PSTI medium at typ ical RH for HVAC environments, (ii) volume to surface ratios are much larger than in the food bags, and (iii) occu pation of such spaces should elevate RH and accelerate kill, a significant potential decrease in airborne transmission of infection might be realized by incorporation of this technol ogy into HVAC filters. Iodine overexposure from the medium tested would be highly unlikely given the enormous dilution factor inside the structure or shelter.

We submit that through space capture of iodine from the triiodide complex has been verified but shown to act too slowly to be useful for individual protection; however, a role for collective protection appears possible. Addi tional study is appropriate repetition in an unreactive container will allow exact measurement of half lives, and larger sample sets will provide more statistical confidence in the values measured. A more robust microbe, possibly *Mycobacterium tuberculosis*, should be evaluated by this or a similar method using a less efficient filter medium and, to establish clinical relevance, in an animal model.

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Conflict of interest

No conflict of interest declared.

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