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Technical note

Use of dialdehyde starch treated filters for protection against airborne viruses

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Abstract
In the event of a pandemic, the general public would use filters as protective devices. However, most commercial filters only remove airborne viruses physically without inactivating them, allowing reproduction on the surface and yielding the mask as a fomite. The objective of this study was to investigate the inactivation performance of dialdehyde starch (DAS) treated filters against airborne viruses.

The viable removal efficiency by and relative survivability on the biocidal filters prepared with dialdehyde starch compared to untreated filters were investigated using MS2 bacteriophage at high relative humidity (80–90%) and room temperature. Experimental results showed no significant difference in viable removal efficiency and pressure drop between the treated and untreated filters for polypropylene filtering facepiece respirators. The pressure drop of DAS treated cellulose filters significantly decreased although there was no significant change in viable removal efficiency; the combination of these two factors resulted in an increase of filter quality. All biocidal filters showed a significantly lower relative survivability than untreated filters, and the relative survivability decreased as the concentration of DAS increased. The biocidal filter treated with 4% DAS presented an average of 30% survivability compared to the baseline of untreated filters. The results demonstrate that dialdehyde starch can be incorporated onto filters to provide an effective means for inactivating MS2 viruses through surface contact.

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1. Introduction
The increasing threat of pathogenic virus outbreaks such as Severe Acute Respiratory Syndrome (SARS), avian flu, and the more recent swine flu have spurred the public’s concerns in regards to the health related issues and protection methods against viral aerosols (CDRF, 2006). Filters are one of the most commonly used devices to remove viral aerosols because of their affordability, ease of application, and effectiveness (Fisher et al., 2009). Filtration efficiency is determined by several mechanisms such as interception, impaction, diffusion, gravity, and electrostatic attraction, depending on fiber density, diameter, filter thickness, and other factors (Hinds, 1999). However, these mechanisms are only related to the physical capture of viruses onto the substrate. Conventional filters still allow these collected viruses to infect others if reaerosolized through the respiratory processes of exhalation, sneezing, or coughing, or as a fomite (CDRF, 2006).
Filters can be modified by incorporating chemicals as antimicrobial agents to inactivate viruses (Gabbay et al., 2006; Silver et al., 2006; Lee et al., 2009). Aldehydes such as formaldehyde and glutaraldehyde are also well known antimicrobial agents, as they are highly reactive molecules which are able to combine with proteins and nucleic acids of microbes by crosslinking and alkylation. Nevertheless, their use as incorporated agents has been limited in the air filtration field because of the toxic effects if small aldehyde compounds were to be released and inhaled (Mcdonnell & Russell, 1999). For this reason, polymeric dialdehydes, such as dialdehyde starch (DAS), are promising alternatives. They not only react with hydroxyl, amino, imino, and sulfurhydryl functional groups, resulting in the inactivation of viruses, but they also show very low toxicity (Mcdonnell & Russell, 1999; Radley, 1976). Our preliminary study demonstrated that a DAS aqueous suspension acted as an effective biocide against MS2 bacteriophage, PRD1 bacteriophage, and poliovirus. Thus, it is of great interest to employ DAS to modify filters for effective and affordable protection against viral aerosols.

The objective of this study was to explore the inactivation efficiency and filtration performance of commercial filters modified by different DAS suspension concentrations. Three different types of filters, namely, two cellulose filters (CFs) that are commonly used for air cleaning and a polypropylene filtering facepiece respirator (PF), were modified with DAS aqueous suspension. Porosity of CFs was factored into the filter selection due to their influence on collection efficiency of viral aerosols.

2. Materials and methods

2.1. Test filters

DAS aqueous suspension was prepared by mixing DAS (granular form, Sigma-Aldrich, P9265) with deionized (DI) water and then cooked at 95°C for 2 h, as described in Song et al. (2009). After heat treatment to allow the granules to release the DAS polymeric molecules (Veelaert et al., 1997), gel formation was observed.

Coarse pore cellulose filters (CCFs, Whatman No. 54 with a thickness of 185 μm and a pore size of 22 μm), fine pore cellulose filters (FCFs, Whatman No. 50 with a thickness of 115 μm and a pore size of 2.7 μm), and facepiece respirators made of polypropylene (PFs, Dupont 01-361-N) were employed in this study. Test filters were prepared by immersing filters into 100 mL of the suspension with different concentrations (i.e., 1%, 2%, 3%, and 4%) for 5 min. These filters were immersed in the same volume of DI water overnight and then washed several times with DI water to rinse out excess DAS. After removing the residuals, the filters were dried at room temperature overnight. In this study, 4% was selected as the maximum concentration because in preliminary test, DAS treated filters with higher concentrations were fragile, indicating unsuitability for air filtration.

2.2. Test agent

MS2 bacteriophage (MS2; ATCC15992-B1™), which is a commonly used surrogate for human pathogen enterovirus (e.g., rotavirus) due to their similarities in resistance to antimicrobial agents, was used as the test agent. MS2 is easy to prepare and assay, and it only requires a bio-safety level 1 facility. Freeze-dried MS2 was suspended in sterile DI water and diluted to a titer of $10^8$–$10^9$ plaque forming unit (PFU)/mL. Artificial saliva was used as the nebulization medium to emulate aerosols produced from sneezing or coughing. Details of the recipe for artificial saliva are reported in Woo et al. (2010).

2.3. Experimental method

A schematic diagram of the experimental set-up for aerosol filtration is displayed in Fig. 1. MS2 aerosol was generated by a six-jet Collison nebulizer (Model CN25, BGI Inc.) that had a virus titer of $10^8$–$10^9$ PFU/mL at a flow rate of 7 Lpm with a pressure of 6 psi. The flow rate through the system was controlled by a rotameter, which was calibrated with a primary...
flow meter (DryCal®, DC-Lite, Bio International Co.). The flow containing MS2 aerosol joined the other flow rate of 7 Lpm, passed through a humidifier for RH control, and then proceeded towards a 2.3-L glass mixing chamber. Thereafter, the flow was split and each flow then reached the corresponding filtration unit at a flow rate of 3 Lpm. This flow rate corresponds to the face velocity of 14.2 cm/s, which is a standard value for respirator filtration testing (NIOSH, 2005). The excess air passed through a separate line and bypassed the test filter. The viral aerosols that penetrated the test filter (Φ=22 mm) were collected by BioSamplers (SKC Corp.) containing 15 mL of sterile DI water at a total flow rate of 5.5 Lpm for 30 min for the experimental line. The reason for using a lower flow rate than the standard 12.5 Lpm is to avoid significant reaerosolization of MS2 at high flow rate (Riemenschneider et al., 2010). An empty filter holder without a filter was used for the control line. The collected sample was assayed with Escherichia coli as a host by the single layer method (EPA, 1984).

The viable removal efficiency (VRE) was determined by comparing the PFUs from the experimental and control BioSamplers:

\[
\text{VRE} = \left( \frac{N_c - N_e}{N_c} \right)\tag{1}
\]

where \(N_c\) and \(N_e\) are the viral concentrations collected by the control and the experimental BioSamplers, respectively. To quantify the amount of MS2 virus collected in a given filter, the test filter taken off from the system was immersed into 25 mL of sterile DI water as an eluent and shaken by a wrist action shaker (Model 75, Burrell Scientific) for 15 min. To evaluate the biocidal efficacy of treated filters compared to untreated filters, relative survivability (RS) of viruses on filters was calculated by comparing survival factors, SFs, of both filters as

\[
\text{RS} = \frac{\text{SF}_{\text{treated filter}}}{\text{SF}_{\text{untreated filter}}}\tag{2}
\]

where \(\text{SF}\) is the viral concentration obtained from the eluent, \(\phi_{VE}\) is the extraction efficiency of microbes from filter, \(\phi_{CE}\) is the collection efficiency of the Biosampler, and \(V_E\) and \(V_B\) are the liquid volumes of the eluent and the Biosampler, respectively. Consideration of the extraction efficiency and the collection efficiency of the BioSampler was not necessary for this relative value, RS. The pressure drop across the filter was measured by a Magnehelic gauge to evaluate filter performance using quality factor (QF), a useful criterion for comparing filter performance of different filter types and thicknesses. This value is defined as (Hinds, 1999)

\[
\text{QF} = \frac{-\ln(1 - \text{VRE})}{\Delta P}\tag{4}
\]

where \(\Delta P\) is the pressure drop. It should be noted that throughout the duration of experiment (30 min), the variation of the pressure drop was negligible. Experiments were carried out in triplicate and samples were assayed in duplicate. Statistical analysis was conducted using 2-way analysis of variance (2-way ANOVA) with Design-Expert® 8.0 software. Additionally, a scanning electron microscope (SEM, JEOL JSM-6330F, JEOL Inc.) was used to observe morphological changes of the filters.

3. Results and discussions

CCFs and FCFs slightly shrank after the DAS treatment, whereas no visible change was observed for PFs. Also, when CFs were subjected to the wrist action shaker, untreated filters were broken after 15 min whereas treated filters remained intact. This is presumably because of crosslinking or entanglement of DAS with the cellulose. SEM images of untreated and treated filters with different DAS suspensions, as shown in Fig. 2, confirm this hypothesis. CFs are composed of main fibers intact. This is presumably because of crosslinking or entanglement of DAS with the cellulose. SEM images of untreated and treated filters with different DAS suspensions, as shown in Fig. 2, confirm this hypothesis. CFs are composed of main fibers intact. This is presumably because of crosslinking or entanglement of DAS with the cellulose. SEM images of untreated and treated filters with different DAS suspensions, as shown in Fig. 2, confirm this hypothesis. CFs are composed of main fibers intact. This is presumably because of crosslinking or entanglement of DAS with the cellulose. SEM images of untreated and treated filters with different DAS suspensions, as shown in Fig. 2, confirm this hypothesis. CFs are composed of main fibers intact. This is presumably because of crosslinking or entanglement of DAS with the cellulose. SEM images of untreated and treated filters with different DAS suspen

The pressure drop results are listed in Table 1. Both filter type (\(p < 0.0001\)) and DAS concentration (\(p < 0.0001\)) are significant as well as the interaction between filter type and DAS concentration. Since the pressure drop of FCFs at 3 Lpm was out of the measurable range, the pressure drop at 0.5 Lpm was measured and then converted for 3 Lpm by

\[
\Delta P_2 = \left( \frac{\Delta P_1}{V_2} \right) \times V_1\tag{5}
\]

where \(V\) is face velocity. The pressure drops of untreated and DAS treated PFs were less than 1 in. H₂O at a face velocity of 14.2 cm/s. These values were much lower than the inhalation resistance of 2.52 in. H₂O permitted by NIOSH for certified respirators (NIOSH, 2005) and the military standard of 4.21 in. H₂O for HEPA filter media (U.S. Army, 1998). Furthermore,
there was no significant change in pressure drop as a function of DAS treatment. The pressure drop of untreated CCF was 3.45 in. H₂O, which is not suitable for the application of a respirator. However, those of treated CCFs ($p < 0.0001$) significantly decreased with increases of DAS concentration. Finally, those treated with the 4% DAS suspension reached the NIOSH limit for respirators. Meanwhile, the pressure drop of untreated FCF was too high to use as a ventilation filter and respirator, but those of the treated FCFs ($p < 0.0001$) significantly decreased although the pressure drops were still over the criterion for respirators.

**Table 1**

| Concentration of DAS suspension (%) | Pressure drop (inch H₂O) |  |
|------------------------------------|--------------------------|--|
|                                    | PF                       | CCF | FCF* |
| 0                                  | 0.69 ± 0.01              | 3.45 ± 0.00                          | 32.40 ± 0.76 |
| 1                                  | 0.69 ± 0.01              | 3.25 ± 0.07                          | 27.00 ± 1.27 |
| 2                                  | 0.64 ± 0.01              | 3.15 ± 0.00                          | 25.92 ± 0.01 |
| 3                                  | 0.65 ± 0.00              | 2.95 ± 0.07                          | 8.10 ± 0.21  |
| 4                                  | 0.63 ± 0.04              | 2.53 ± 0.04                          | 3.24 ± 0.00  |

* Converted from 0.5 Lpm.

**Fig. 2.** SEM images of untreated (A) PF, (D) CCF, and (G) FCF, treated (B) PF, (E) CCF, and (H) FCF with 2% DAS suspension, and treated (C) PF, (F) CCF, and (I) FCF with 4% DAS suspension. Magnifications of (A)-(C) 500 × and (D)-(I) 1000 ×, respectively.
treatment reduced air resistance as pressure drop decreased. Accordingly, an increase in the QF was observed. The QFs of untreated and 4% DAS treated CCFs were 0.77 and 1.46 kPa/C0 and those of FCFs were 0.25 and 1.85 kPa/C0, respectively. The impact on FCF (e.g., increase of 7.4 times) was larger than on CCF (e.g., increase of 1.9 times). The amount of crosslinking of the fibrillates is likely responsible for the difference.

To evaluate the inactivation capability of DAS treated filter mats, the RS of treated filters as a function of concentrations of DAS suspension is displayed in Fig. 4. All filters show similar tendencies, with RS decreasing with increasing concentration of DAS suspension, indicating the inactivation effect of the DAS treated filters against the MS2 collected on the treated filter. Aldehyde functionality of polymeric molecules dispersed from gel formation through heating in preparation is the source attributed to the biocidal capacity on treated surfaces (Song et al., 2009).

4. Conclusions

This study demonstrates that treating filters with inexpensive DAS (less than $1/10 filters) is a practicable method for creating biocidal filters. DAS treatments have different impacts on filtration efficiency depending on filter materials. The treatment reduces air resistance in cellulose filters, resulting in lower pressure drop while having no significant impact on removal efficiency. These factors combine to yield an improved quality factor for cellulose filter. Meanwhile, there is no difference in quality factor for treated propylene filters. Nevertheless, the biocidal effect is clearly exhibited for all treated filters. Compared to other decontamination methods, the DAS treatment does not require additional energy sources and facilities (e.g., microwave, IR, and UV irradiation) and it does not release toxic chemicals (e.g., iodine treated filter). Therefore, the treated filters can be adopted for wide applications such as in health care facilities and at pandemic events.
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