Demonstration of Hollow Fiber Membrane-Based Enclosed Space Air Remediation for Capture of an Aerosolized Synthetic SARS-CoV-2 Mimic and Pseudovirus Particles

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ABSTRACT: Reduction of airborne viral particles in enclosed spaces is critical in controlling pandemics. Three different hollow fiber membrane (HFM) modules were investigated for viral aerosol separation in enclosed spaces. Pore structures were characterized by scanning electron microscopy, and air transport properties were measured. Particle removal efficiency was characterized using aerosols generated by a collision atomizer from a defined mixture of synthetic nanoparticles including SARS-CoV-2 mimics (protein-coated 100 nm polystyrene). HFM1 (polyvinylidene fluoride, ~50–1300 nm pores) demonstrated 96.5–100% efficiency for aerosols in the size range of 0.3–3 μm at a flow rate of 18.6 ± 0.3 SLPM (~1650 LMH), whereas HFM2 (polypropylene, ~40 nm pores) and HFM3 (hydrophilized polyether sulfone, ~140–750 nm pores) demonstrated 99.65–100% and 98.8–100% efficiency at flow rates of 19.7 ± 0.3 SLPM (~820 LMH) and 19.4 ± 0.2 SLPM (~4455 LMH), respectively. Additionally, last filtration with minimal fouling was demonstrated using ambient aerosols over 2 days. Finally, each module was evaluated with pseudovirus (vesicular stomatitis virus) aerosol, demonstrating 99.3% (HFM1), >99.8% (HFM2), and >99.8% (HFM3) reduction in active pseudovirus titer as a direct measure of viral particle removal. These results quantified the aerosol separation efficiency of HFMs and highlight the need for further development of this technology to aid the fight against airborne viruses and particulate matter concerning human health.

KEYWORDS: PM2.5, SARS-CoV-2, COVID-19, indoor air, bioaerosol

INTRODUCTION

The key role of airborne transmission of COVID-19 in the rapid expansion and widespread nature of the current pandemic has highlighted the need for highly effective, low pressure filter technologies to remove viral aerosols in indoor environments like restaurants and hospitals.1 The emergence of more transmissible viruses like the Delta variant further emphasizes the value of controlling respiratory spread. Viral aerosols (droplets <5 μm) are created by medical procedures, eating, coughing, sneezing, and even normal breathing, and these aerosols as well as larger droplets contribute to viral transmission. Aerosols containing pathogens like SARS-CoV-2 have been identified in a number of studies (reviewed in ref 9), further establishing the need for cost-effective and efficient air purification technologies in this and future respiratory pandemics. In addition to bioaerosols, there is a great need for effective technologies to remove particulate pollution from air given the known detrimental health effects of exposure to particulate matter.12–15 Furthermore, it has been suggested that PM2.5 (airborne particulate matter generally 2.5 μm or smaller) may act as a carrier for transmission of viral aerosols16 and even that PM2.5 exposure increases the risk of severe COVID-19, reaffirming the need of aerosol filtration in mitigating the spread of airborne pathogens as well as protecting human health from various airborne particulate matter.

Porous, thin membranes with asymmetric pore structure offer several features that may be advantageous for aerosol filtration.19 Polymer materials can be easily modified for surface functionalization to tune surface properties of the membrane or add a new functionality such as enzymes or nanoparticles.20–22 Easy control of thickness, pore size and structure, and porosity allows for tuning the size cutoff for a given application, i.e., filtration of viruses with defined size, by controlling transport properties to minimize the pressure drop while maintaining high efficiency filtration. The wide range of tunable features may provide significant advantages. Asymmetric pore structures, for example, can provide highly efficient filtration (via sieving) with small pore sizes at the feed surface,

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while minimizing the pressure drop with widening pore structure below (Figure 1A). In this orientation, fouling may be minimized by the sieving separation mechanism since particles are unable to enter the filter media and clog air transport pathways (Figure 1B). Moreover, the mechanism of aerosol separation can be tuned by changing the configuration of air flow through an asymmetric membrane, i.e., sieving separation at the porous membrane surface in one direction versus capture within the porous structure in the other (Figure 1B,C). The ability to capture particles within the porous network may also have potential advantages for sensing and quantifying certain captured components of interest such as pollutants or pathogens.

Hollow fiber membranes (HFMs) are a particular type of membrane with cylindrical geometry where transport occurs across the membrane in the radial direction. HFMs are highly advantageous for high-throughput scenarios since their geometry allows for high surface area to volume ratio, packing large filtration areas into a small footprint, and for low pressure operations. Due to these advantages, HFMs have been applied extensively in the water purification and membrane distillation areas where a high surface area is needed to account for the low flux through highly selective membranes like those used in reverse osmosis or membrane distillation.

Only a few studies have investigated the use of HFMs for aerosol filtration; however, these have shown great promise for separating PM$_{2.5}$ from air. Several studies have demonstrated efficient aerosol filtration with hollow fiber membranes, often with combustion products as the test aerosol similar to the biomass burning-derived secondary organic aerosols (SOA), which are a major contributor to anthropogenic PM$_{2.5}$. Furthermore, some have shown easy regeneration/reuse of HFMs in filtering aerosols. To our knowledge, no studies have demonstrated viral aerosol capture using HFM modules with differing properties. Efforts to design filters and methods for removing/deactivating viral aerosols have increased in light of the current coronavirus pandemic. We sought to use commercially available HFMs (polypropylene, PVDF, and polyether sulfone) for quickly deployable and effective aerosol filtration to curb the spread of the COVID-19 pandemic rather than developing new membranes or other novel solutions that will take time to reach the public.

In this work, the overall objective was to quantify air and particle transport in commercially available HFM modules for aerosol filtration to remove viral aerosols and other airborne particles as a quickly deployable and cheaper alternative to HEPA systems with higher aerosol removal efficiency than standard HVAC filtration. Two commercial membranes and one noncommercial production-scale membrane were chosen with varying characteristics (pore size, shape, and asymmetry; membrane thickness; material hydrophobicity; and module design) to establish relationships between membrane features and aerosol filtration functionality. Membranes from these modules were characterized by porosimetry and scanning electron microscopy. The modules’ effective filtration of aerosol mixtures of controlled sizes, including protein-labeled nanoparticle mimics of SARS-CoV-2, was confirmed. The real-world applicability for each module was evaluated by longer term (two day) filtration experiments with ambient aerosols. Finally, the HFM modules were evaluated with aerosolized vesicular stomatitis pseudovirus particles with filtration efficiency characterized by viral titering assays, demonstrating their value for reducing airborne respiratory illness spread.

### MATERIALS AND METHODS

**Aerosol Testing System Construction.** The membrane-based aerosol testing system was constructed as shown in Supporting Information Figure 1. Tubing was mostly constructed of PTFE 1/2 in. O.D. tubing (McMaster Carr) with some exceptions noted in the schematic. All connections and valves were brass or stainless steel (Mcmaster Carr). Aerosol size distributions were measured in an ~3 in. I.D. PTFE tube (constructed of a rolled and taped skived PTFE sheet from Mcmaster Carr) that served as a depressurizing chamber to avoid pressure damage to the pump in the optical particle counter (MetOne Instruments GTS265). Mass flow

![Figure 1. Schematic representation of pore structure role in aerosol filtration.](image)
Table 1. Summary of Properties Collected for Hollow Fiber Membrane Modules Tested in This Work

|                  | HFM1          | HFM2          | HFM3          |
|------------------|---------------|---------------|---------------|
| membrane material| polyvinylidine fluoride (PVDF) (hydrophobic) | polypropylene (PP) (hydrophobic) | hydrophilized polyether sulfone (hPES) (hydrophilic) |
| module packing fraction | 0.34          | 0.47          | 0.48          |
| mean pore size   | 1153 nm (mercury porosimetry) | 40 nm (manufacturer) | 200 nm (manufacturer) |
| shell surface mean pore size | 57 ± 35 nm | 42 ± 17 nm | 140 ± 87 nm |
| lumen surface mean pore size | 1346 ± 1086 nm | 46 ± 27 nm | 748 ± 896 nm |
| bulk porosity    | 24% (Hg porosimetry) | 40% (manufacturer) | 45% (SEM cross-section pore analysis) |
| tortuosity       | 3.1692 (Hg porosimetry) | not measured | not measured |
| thickness        | 412.6 ± 106.2 μm | 41.8 ± 2.2 μm | 85.5 ± 3.5 μm |

“Data collected in this work are shown in Supporting Information Figures 11–25.”

Rates were measured by a thermal mass flow meter (Model 4043, TSI), and the transmembrane pressure differential was measured using a digital manometer (0–100 psi, SPER Scientific).

Membrane Structural Characterization. The module packing fraction was estimated as the ratio of the fiber count times fiber cross-sectional area divided by shell cross-sectional area. Hollow fiber membrane samples (including tribore HF) were prepared for scanning electron microscopy by mounting on EM conductive carbon tape (Nissin) and (in most cases) sputter coating with 5 nm platinum (Leica EM ACE600). Imaging was performed in the University of Kentucky Electron Microscopy Center using FEI Helios Scanning Electron Microscope (SEM). Detailed preparation methods for each sample are provided in Supporting Information Detailed Methods. Nitrogen porosimetry (Micromeritics Tristar 3000) was also performed for HFM2 to validate other measurements determined by SEM (Table 1).

Air Permeability Characterization. For all permeability experiments, the air was supplied from a filtered regulator set at ~4.1 bar attached at two building air outlets, with feed pressure further controlled by the needle valves at each outlet. The filtered air generally has a baseline residual level of particles of ~300 per liter for 300–500 nm particles. For air permeability experiments, the setup in Supporting Information Figure 1 was modified to take the atomizer out of the system. The needle valves at the filter regulators were used to control the pressure drop across the module, and the air flow rates, temperatures, and pressure drop readings were recorded. The measured mass flow rates were then normalized by total surface area of the module, as estimated by methods detailed in Supporting Information Detailed Methods.

Filtration Efficiency Assessment. Aerosols were generated using a constant output collision atomizer (TSI model 3076) fed from a mixture of 50 nm lipopic acid-coated gold nanoparticles (NanoComposix), protein-labeled (10:1 GFP:Spike) 100 nm COOH-functionalized polystyrene latex nanoparticles (Bangs Laboratories, Inc.), and 500 nm amine-functionalized polystyrene latex nanoparticles (Polysciences). The mixture was characterized via dynamic light scattering (Anton Paar Litesizer 500) before each aerosol experiment (Supporting Information Figure 2). The protein-labeled 100 nm PSL nanoparticles were prepared by first coating particles with Ni2+ followed by labeling with either a superfolder green fluorescent protein (GFP) or a spike protein with polyhistidine tags, with labeling verified by a change in hydodynamic diameter measured by DLS and stability of immobilization later confirmed by Bradford and SDS-PAGE analyses; unfortunately, the spike protein was found to be degraded after several months of storage, and therefore we can only confirm that 90% of the 100 nm particles were protein-labeled with a GFP (Supporting Information Figure 3). Detailed methods can be found in the Supporting Information. Aerosol concentrations were measured using an optical particle counter (Met One Instruments GT-526S), operated in differential mode to show individual totals for each default size bin (0.3–0.5 μm, 0.5–0.7 μm, 0.7–1 μm, 1–2 μm, 2–3 μm, >3 μm). At each time point data were collected by first measuring the aerosol concentrations and then switching the outlet to the flow meter for pressure drop/flow rate measurement. Next, the bypass valve was switched to circumvent the filter, and the pressure drop/flow rate measurement was taken before switching the outlet valve back to the sampling tube. The unfiltered stream flushed the sampling tube for 1–3 min before taking the unfiltered aerosol concentration measurement (Cunfiltered). Thus, matching unfiltered/filtered concentrations are available for each time point, as well as pressure drop (ΔP) and flow rate data for each. Filtration efficiency was calculated as a percentage by eq 1, and the quality factor was calculated by eq 2:

\[ \eta = \frac{C_{\text{unfiltered}} - C_{\text{filtered}}}{C_{\text{unfiltered}}} \times 100 \]  

\[ \text{QF} = \frac{-\ln(1 - \eta/100)}{\Delta P} \]  

For long-term filtration studies, the system was operated with a vacuum pump driving filtration of ambient aerosols, with the system set up as shown in Supporting Information Figure 4. In this case, a Grimm miniWRAS 1371 aerosol spectrometer was used to characterize particle size distributions, which provides size distribution and concentration information for particle diameters from 10 nm to 193 nm using an electrophoretic mobility sizer with electrometric detection and from 253 nm to 35 μm using an optical scattering detector. Occasional outliers in measurements of particle counts were identified as >3 standard deviations from the mean over the measurement time range for each filtered and unfiltered point, and uncertainty was propagated to the value of efficiency calculated from the corresponding time-averaged counts.

Pseudovirus Production. The purification process is depicted in Supporting Information Figure 5. HEK 293T cells
were cultured in DMEM + 10% FBS. Transfections of 293T cells, with VSV G protein, took place in 10 cm dishes, with 8 μg of plasmid DNA, using Lipofectamine 3000 reagents (Life Technologies L3000075), and incubated for 24 h at 37 °C and 5% CO₂. Cells were then transduced with VSV ΔG-GFP genome pseudovirus, incubated for 1 h, washed 2× with phosphate buffered saline (PBS), and incubated for 24 h. Supernatants were collected, frozen in a dry ice methanol bath, and stored at −80 °C. Samples were purified on a 20% sucrose cushion in ultracentrifuge rotor SW28, for 2 h at 27 000 rpm and 4 °C. Pseudovirus was resuspended in 10% sucrose in 1× TNE (50 mM Tris-HCl, 150 mM NaCl, and 1 mM EDTA), rocking at 4 °C overnight. Pseudovirus was pooled, frozen using a dry ice methanol bath, and stored at −80 °C.

Pseudovirus Stocks. Pseudovirus stocks were thawed and diluted to 1:2 into HyClone Dulbecco’s Modified Eagle Medium (DMEM) and then placed on ice during transport. The pseudovirus stock was loaded into a syringe, and the pump was set for 0.1 mL/min, consistent with the expected feed rate for the atomizer in recirculation mode that was used for nonbiological filtration tests. Aerosols were collected by a custom-made impinger comprised of a cell culture flask with 5 mL DMEM (Cytiva, cat. no. sh30022.01) and a serological pipet as a nozzle (Supporting Information Figure 8). After 5 min (delivery of 0.5 mL of pseudovirus solution, ~2.7 × 10⁷ particles), the pump turned off, and the air supplies were left on for 1–2 min to flush remaining aerosolized pseudoviruses through the filter and system; then, the air supplies were also turned off. The described method was used for each HFM, and a piece of PTFE tubing of equivalent length was used in place of any HFM test module for the control. Collected aerosols for each HFM and control were concentrated by centrifugation at 3260 g and 4 °C for 4 min using a 100 kDa MWCO centrifugal ultrafiltration cassette (Pall) and tested by pseudovirus transduction assays.

Pseudovirus Transduction Assay. Stable ACE2 expressing HEK 293T cells were seeded in 24-well plates and transduced the following day with pseudovirus. Serial 10-fold dilutions were performed in a fresh 24-well plate, starting with 5 μL of pseudovirus in DMEM+10% FBS, and 300 μL per well

Figure 2. Comparison of hollow fiber membranes by SEM imaging. Cross-section images of each membrane demonstrate the asymmetric pore structures in (A) HFM1 and (G) HFM3 and symmetric pores in (D) HFM2. HFM1 demonstrates highly asymmetric pore structure, with (B) small <100 nm pores at the shell surface and (C) larger pore openings >1 μm at the lumen surface. In contrast, HFM2 has highly symmetric pores <100 nm at both (E) the shell and (F) the lumen surfaces. HFM3 has a moderately asymmetric structure, with (H) small ~140 nm pores at the shell surface and (I) wider ~750 nm pores at the lumen surface.
was transferred to an aspirated well of stable ACE2 293T cells. Transductions were incubated for 24 h, before being visualized on the Axiovert 200 M 5× objective fluorescent channel. Transduced (GFP-expressing) cells were counted, and the pseudoviral titer was calculated using the dilution factor. Samples were transduced in duplicate and were generally highly reproducible between technical replicates and independent biological experiments (see Supporting Information Figure 9).

Statistical analysis was carried out in R Studio (code available upon request). A one-way ANOVA identified statistical differences (p < 0.01) among the group of four samples (unfiltered control, HFM1, HFM2, HFM3), as represented by the mean titer value of each biological experiment (N = 3), scaled to the undiluted starting titer for each experiment to account for variations in the starting titer for each experiment. Posthoc analysis was performed with Tukey’s Honest Significant Difference test comparing each group using the same scaled titer values, with the resulting statistical significance cutoffs shown in Figure 7.

RESULTS AND DISCUSSION

In this study, the objective was to establish hollow fiber membrane modules with various structural and design features and polymer types as viral aerosol filters to reduce the airborne spread of pathogens. A schematic summary of this study is shown in Supporting Information Figure 10. We first establish predictive models (with comparison to experimental measurements) of air transport properties for each HFM. Next, we demonstrate efficient separation of synthetic (polystyrene and gold) aerosols of various sizes (including SARS-CoV-2 mimicking 100 nm polystyrene nanoparticles with protein surface coating). We demonstrate real-world applicability for HFMs by performing long-term filtration studies with ambient aerosols showing minimal pressure drop changes over time. Finally, we connect the observed separation of synthetic virus mimics with functional tests of pseudovirus removal via infectivity assays, confirming that HFMs are viable for enclosed space air filtration to help reduce the spread of airborne respiratory illnesses. To our knowledge, this work represents the first demonstration of hollow fiber membranes for viral aerosol filtration.

Selection of Hollow Fiber Membranes. In this work, three hollow fiber membranes (HFM1, tribore fibers from START Centre Singapore; HFM2, X50 membrane from 3M; and HFM3, Lifeslaw from Vestergaard) were chosen for characterization and quantification as aerosol filters. These membranes have quite variable properties, from surface characteristics to pore size and uniformity. HFM1 has a highly asymmetric, larger pore structure that is spongy and open on the lumen side with smaller pores and denser structure on the shell side (Figure 2B,C, Table 1, Supporting Information Figures 11−13) and also has a unique tribore geometry (Supporting Information Figure 12) that may allow for higher diffusive capture due to increased surface area and higher pressure (i.e., more air processing capability) due to increased mechanical strength. On the other hand, HFM2 has more uniform surface pore structures (about 40 nm diameter) with porosity and pore sizes matching much more closely for the shell and lumen side (Table 1, Figure 2E,F, Supporting Information Figures 14−16). In addition to the more symmetric structure across the membrane thickness, this membrane has internal pore networks that are more fibrous than spongy (Figure 2D, Supporting Information Figure 15), which may be a more similar structure to fibrous filters commonly used in air filtration. HFM3 has an asymmetric pore structure and a more fibrous internal network, thus combining two key features of HFM1 and HFM2 for a broader survey of functional characteristics with variable membrane features. Additionally, HFM3 has an apparently higher porosity than the other two membranes (Figure 2G). Notably, PTFE hollow fiber membranes with fibrous pore networks have previously shown success as aerosol filters, albeit with less than ideal efficiency (~90%) for 300 nm particles.

In addition to pore structure variables, the HFMs in this work were chosen to sample different material properties for a more comprehensive characterization of key parameters for membrane-based air filtration. For example, HFM1 and HFM2 are both constructed of highly hydrophobic materials (polyvinylidene fluoride PVDF and polypropylene PP, respectively), whereas HFM3 has a hydrophilic surface property. Importantly, these differences in surface chemistry may have a meaningful effect on function in aerosol filtration, especially with respect to virus neutralization by immobilizing on the surface, since it has been demonstrated that hydrophobic surfaces can contribute to virus deactivation (see also Supporting Information Figure 7). Furthermore, a previous study has shown that hydrophobicity of the membrane can affect the fouling properties of membranes used for aerosol filtration of hygroscopic particles, suggesting further study of membrane properties may inform future work designing membranes for aerosol filtration. Importantly, all three materials can tolerate disinfection via 70% ethanol, a common disinfectant available to the general public.

Hollow Fiber Membranes Present Advantageous Pore Features for Aerosol Filtration. The three hollow fiber membranes (HFM) were characterized initially to determine their potential for aerosol filtration (Table 1). In particular, detailed knowledge of the pore structure is advantageous for understanding and predicting the capture of aerosols. Features such as tortuosity (τ), pore diameter (rp), overall porosity (ε), and membrane thickness (δ) govern flux (J) across the membrane (see eq 3), and therefore knowledge of these parameters can guide the choice of membrane for a particular application.

\[ J \propto \frac{\varepsilon \times r_p}{\delta \times \tau} \quad (3) \]

Furthermore, tortuosity and pore asymmetry likely affect the capture or separation of particles from air. For example, the increased path length associated with higher tortuosity increases diffusion mediated capture for smaller particles. On the other hand, since increased air velocity increases impaction, asymmetric pores with bottlenecks could increase efficiency for larger particles’ filtration. Therefore, several techniques were employed to characterize membrane properties.

Scanning electron microscopy (SEM) was used to examine membrane pores in detail (Figure 2, Supporting Information Figures 11−19), revealing variable properties among the three tested membranes. Determination of tortuosity by imaging is not easy; however, examination of several cross-section views by SEM suggests highly branched networks of pores within the membranes. In the case of HFM1, a highly branched, porous network with spongy structure is observed near the lumen, while the shell surface was found to have smaller pores with much lower porosity (Table 1).
The HFM1 fiber was found to have a highly asymmetric pore structure, with significantly wider pore openings in the lumen surface than in the shell surface (∼57 nm vs ∼1.3 μm, Supporting Information Figure 11C vs 13C). A similar asymmetric pore structure was also observed in the HFM3 membrane (∼140 nm vs ∼750 nm, Supporting Information Figure 17C vs 19B), while the HFM2 fibers were notably less variable in pore size at the lumen versus shell surfaces (∼40 nm on each side, Supporting Information Figure 14B vs 16C). The result of the imaging analysis suggests that the pore asymmetry of the HFM1 and HFM3 fibers may be make them ideal candidates for more versatile application in air filtration given the potential to tune performance by controlling pore structure (Figure 1).

In addition to SEM, nitrogen porosimetry was also used to characterize the membrane structure for the HFM2 membrane. The pore size distribution was unobtainable by porosimetry for HFM1 and HFM3 given the limits of nitrogen as the working fluid; however, for HFM2 the result (average ∼38 nm pore diameter, Supporting Information Figure 20) was reasonably consistent with SEM analysis (∼42−46 nm average pore diameter, Supporting Information Figure 24) and manufacturer specifications. Furthermore, mercury porosimetry data (Supporting Information Figure 21) provided with the generous gift of HFM1 from the START Centre also shows comparable pore distribution to that of SEM analysis (Supporting Information Figure 24).

HFM Modules Demonstrate Predictable Air Transport Properties. As the first step to assessing performance of the three membranes, dead-end mode (all air flow passing through membrane) air permeability was measured for each module (Figure 3). Notably, the flow rate measurements

![Figure 3](https://doi.org/10.1021/acsestengg.1c00369)
normalized by area as a function of transmembrane pressure (Figure 3, panel A) demonstrate the expected pattern of permeability values (equivalent to the slope of the linear regression), with the smallest pore size membranes (HFM2, nominal 40 nm pores) showing the lowest permeability and the largest pore size membranes (HFM3, nominal 200 nm pores) showing the highest permeability. This is as expected, given the known relationship between flux and membrane properties (eq 3). While these modules do require a higher pressure as driving force than standard HEPA filters (generally ∼0.003 bar), previous work has shown that polymer membranes tend to maintain their initial pressure drop longer than fibrous HEPA filters. Nonetheless, these results demonstrate that HFM3 coupled to a vacuum pump or compressor operating at ∼0.5 bar pressure differential would allow filtering the full restaurant air volume every ∼7.7 h for a typical small restaurant (∼20 m × ∼10 m × ∼2.5 m) (see Supporting Information Detailed Methods for information on calculations). For comparison, a typical HEPA filter (operating at ∼0.003 bar) could filter the same air volume every ∼11.9 h.

Zohar et al. derived expressions for the flow of gases through planar microchannels, and adaptation of their solution for cylindrical geometry (see Supporting Information Detailed Methods) results in the following relationship (eq 4)

\[
m_{\text{single pore}} = \left( \frac{\pi r^4}{8 \delta} \right) \frac{P}{RT \mu} (\Delta P)(1 + 8Kn)
\]

where \(m_{\text{single pore}}\) is the mass flow rate through a single pore, \(r\) is the pore radius, \(\delta\) is the membrane thickness, \(P\) is the average absolute pressure in the membrane, \(R\) is the gas constant, \(T\) is the absolute temperature, \(\mu\) is the fluid viscosity, and \(Kn\) is the Knudsen number defined by the ratio of air mean free path \(\lambda\) and pore diameter (\(Kn = \frac{\lambda}{2\delta}\)). Applying this equation for membranes in this study, we find the theoretical predictions agree quite well (Supporting Information Figure 26).

Interestingly, the calculations performed assuming circular pore geometry for HFM2 are more accurate than those using planar geometry, despite the elliptical shape of the pores at membrane surfaces, which suggests that circular pore geometry can be assumed for most membranes in this application (Supporting Information Figure 26B). Additionally, comparison of two models for HFM1 demonstrate that consideration of the tight shell surface layer alone is adequate for predicting total flux through the membrane, without the need to consider the more porous/larger pore region of the membrane thickness (Supporting Information Figure 26A). In all cases, the agreement between predicted and measured values provides a basis for predicating functionality of membranes designed for aerosol capture in future work.

Importantly, the direction of air flow (shell-to-lumen vs lumen-to-shell) in each membrane has a minimal effect on the permeability (Figure 3). Given the opportunity to control filtration mechanisms by direction of flow for asymmetric pores (Figure 1), the consistency in permeability regardless of flow direction presents another strong indicator for the value of HFMs as enclosed space aerosol filters. Together, the structural features and transport properties of all three membranes suggest great potential for use as effective aerosol filters even for submicron (submicrometer) particles.

**Filtration Testing with a Defined Aerosol Mixture Including a SARS-CoV-2 Mimic Demonstrates Efficient Particle Removal.** Aerosol filtration tests were performed with size-dispersed nanoparticle mixtures (50 nm, 100 nm, 500 nm) including a SARS-CoV-2 mimic (protein coated 100 nm PSL) to determine the aerosol removal efficiency. The SARS-CoV-2 spike protein is a large trimeric assembly that protrudes from the ∼100 nm virus particle and is responsible for binding the ACE2 receptor on human cells to mediate host cell entry. In this work, a superfolder GFP was immobilized on 100 nm PSL to approximate the presentation of the spike protein on a virus particle surface. As shown in Figure 4, all
three membranes show excellent filtration efficiency in the tested size ranges (particle diameter 0.3–3 μm, the size range most relevant for aerosol virus transmission5,11,56) over the course of approximately 1 h run time. In particular, the HFM2 fibers with about 40 nm pores showed the expected best performance demonstrating >99.65% rejection for all particles 0.3–3 μm. HFM3 showed the next best average performance, with >98.8% rejection for all particle sizes tested here. The higher fiber packing fractions of HFM2 and HFM3 (Table 1) may also contribute to better aerosol filtration observed in these cases. Of course, all membranes will have some outliers in pore size that are larger than expected, which may explain the few particles that come through for all three membranes even at the particle sizes much larger than the average pore size.

The high filtration efficiency observed for all three membranes in this work suggests the potential for these HFM modules to be applied in air purification for viral aerosols and airborne particle separation applications. Air pollution particulate matter of varying sizes has been shown to have significant impacts on human health.13,15,17,57 Moreover, it is expected that climate change will continue to increase the levels of air pollution, including potentially PM 2.5,60,61 emphasizing the importance of air cleaning technologies now and in the future. In this application, all three membranes examined here have great potential for removing inhalable particulate matter in a wide range of sizes (<0.1 μm PM1, <1 μm PM10, <2.5 μm PM2.5, <10 μm PM10) with known detrimental effects on health.57–59

**Extended Filtration Testing with Ambient Aerosol Shows High Efficiency and Minimal Fouling.** In order to demonstrate real-world applicability of HFM for aerosol capture, filtration with ambient air was performed continuously for approximately 2 days for each HFM. Notably, while the short-term filtration tests cover the most relevant particle sizes for aerosol transmission of viruses,5,11,56 viral transmission could still occur via smaller aerosols in the range of 100–300 nm. Therefore, in these experiments, the aerosol distribution was measured for all particles >10 nm to further establish filtration efficiency for all aerosol sizes relevant to viral transmission (>100 nm, the size of a single viral particle). As shown in Figure 5, all three HFM showed consistent performance over 48 h with no significant change in filtration efficiency. Only HFM3 showed a noticeable (but small) reduction in quality factor over the course of 2 days, suggesting that all HFMs (especially HFM1 and HFM2) should have a long effective lifetime as air filters. This observed variation is probably due to the likely differences in the filtration mechanisms for HFM1 and HFM2 compared to HFM3; the shell side pore sizes for HFM1 (∼57 nm) and HFM2 (∼40 nm) therefore would sieving the vast majority of aerosols, whereas HFM3 has a larger pore size at the shell surface (∼140 nm) and allows more aerosols to enter pores and deposit, ultimately reducing the effective pore size over time.

**Pseudovirus Aerosols Are Effectively Removed by Hollow Fiber Membrane Air Filtration.** Interestingly, few membrane aerosol filtration studies have focused on the properties of the aerosol particles. For example, biologically derived aerosols will often contain complex mixtures of water, surfactants, sugars, proteins, and lipids, which may alter their adhesion to filters or surfaces as compared to rigid particles like polystyrene latex which are often used for aerosol filtration studies.62,63 Indeed, previous studies have shown the potential for accumulation of water on protein- and salt-containing hygroscopic particles.64 Wang et al.34 demonstrated that particle hygroscopicity may play a role in pressure drop increases of aerosol membrane filters by leading to water accumulation at the membrane surface that forms films blocking pore openings. Given the importance of particle characteristics, we further confirmed the value of HFMs as enclosed space air filters using a model system consisting of VSV (vesicular stomatitis virus) pseudovirus with a GFP
Figure 6. Schematic representation of pseudovirus production, aerosol filtration studies, and transduction assay. (A) VSV pseudovirus (VSVΔG +G) was produced in HEK293T cells and purified. (B) VSVΔG+G was loaded into a syringe pump to the atomizer, and the resulting aerosol was passed through the HFM module filter (or an equivalent length of tubing with no filter) before collection by DMEM solution bubbling. The collected sample was concentrated by centrifugal ultrafiltration (100 kDa MWCO). (D) Pseudoviral transduction assays were performed, wherein serial dilutions of pseudovirus suspensions from aerosol filtration tests are incubated with HEK293T cells that stably express both the LDLR (VSV glycoprotein receptor) and the ACE2 receptor for SARS-CoV-2, resulting in translation of the GFP reporter gene within the VSVΔG+G genome. Quantification of fluorescent cells by microscopy provide quantitative assessment of active pseudoviral particles. Created with BioRender.com.

Figure 7. HFM removes pseudovirus aerosol particles effectively as an enclosed space air filter. Pseudovirus titer assay results with VSVΔG +G particles demonstrating high efficiency in removing active pseudovirus. Data are presented as log reduction in active pseudovirus relative to the undiluted transduction control. The 3-log reduction in titer for the unfiltered control shows the losses due to dilution, aerosolization, and recapture of aerosolized pseudovirus in the system (see also Supporting Information Figure 9B). HFM2 and HFM3 performed exceptionally, demonstrating at least 3-log reduction in active pseudovirus compared to the unfiltered control, resulting in titers below the limit of detection for our assay. For HFM1, data represent average of N = 3 independent biological replicates and for HFM2 and HFM3, and data represent the average of the lower limit for log reduction (assay limit of detection). Error bars represent standard deviation for actual log reduction values (HFM1) or assay limit of detection. Asterisks indicate statistically significant differences (p < 0.05) comparing the unfiltered control log-reduction to each HFM by ANOVA with Tukey HSD posthoc analysis for pairwise comparison. The dashed line represents the average limit of detection for the titering assays. For raw titer data, see Supporting Information Figure 9.  

As shown in Figure 7, all three HFM modules significantly (p < 0.05) reduced the titer of aerosolized virus as compared to the unfiltered control. Notably, HFM1 with a spongy substructure showed a lower performance (~100-fold reduction, ~99% removal) than HFM2 and HFM3 (both ~1000-fold reduction, ~99.9% removal) in removing active pseudoviral particles from air. Importantly, HFM2 and HFM3 both reduced active pseudoviral levels to below the limit of detection (~333 active particles/mL), suggesting that the actual efficiency for these two modules may be higher, but this speculation is tempered by the technical limitations of assays used in this work. It is also worth noting that the aerosol created from pseudovirus suspensions is likely a polydispersed mixture of salt, sugar, and pseudovirus aerosols, since the buffer conditions required for pseudovirus stability include various buffer components. Therefore, the filtration behavior with polydisperse ambient aerosols in longer term experiments...
may be more representative of efficiency with pseudovirus suspensions. Given the similarity of filtration efficiency for pseudovirus particles, ambient aerosols, and protein-labeled nanoparticles in short-term experiments, it is unlikely that specific composition of the pseudovirus suspension plays a major role in particle removal efficiency.

In this study, the concentration of aerosolized pseudovirus used for HFM testing was relatively high (∼10⁵–10⁷ active particles/m³, estimated from collected unfiltered titer and feed titer, respectively), comparable to a closed room with poor circulation where a high virus load emitting infected individual stays for several hours. Even in this situation, application of HFMs with >99% filtration efficiency would likely have a significant effect on reducing transmission. Given that most situations outside of hospitals likely have relatively low concentrations of virus in aerosols where even surgical masks with ∼30–70% efficiency can reduce transmission rates, the high efficiency separation observed here with HFMs is more than adequate to limit viral spread by aerosols for the majority of real-world situations.

CONCLUSION

This work quantifies the efficacy of microporous hollow fiber membrane modules (with three different structures) as enclosed space air filters for viral aerosol separations using both protein coated PSL particles and active pseudovirus particles. Furthermore, application of commercial microporous HFMs may also find other uses for effective control of indoor air quality given the high efficiency filtration demonstrated here, i.e., as prefilters to lengthen the life span of HEPA air cleaning technologies in highly clean environments like semiconductor manufacturing, especially considering the lack of fouling observed for HFM1 and HFM2 in longer filtration experiments. The higher performances of HFM2 and HFM3 suggest that fibrous internal pore networks may be advantageous as compared to the spongy network of HFM1. These results also lay the foundation for further investigation of HFMs for use in aerosol filtration and, furthermore, provide a framework for design choices in future work developing membrane-based aerosol filtration technologies. For example, our results indicate that reducing the membrane thickness would improve the pressure drop while maintaining high efficiency aerosol separation, since the two thinner membranes (HFM2 and HFM3) had the highest filtration efficiency. Furthermore, long-term filtration experiments also suggest that designing pore structures for sieving separation (i.e., asymmetric pores) may minimize fouling and extend the HFM air filter lifetime while maintaining high efficiency aerosol separation. While the HFMs tested here do not outcompete standard HEPA systems in efficiency or pressure drop, we demonstrate here that commercially available HFMs originally designed for other uses are a highly cost-effective and convenient option for removing viral particles from air for businesses with enclosed spaces (i.e., restaurants, gyms, etc.) to minimize the chances of respiratory illness transmission and mitigate the spread of the COVID-19 pandemic.

ASSOCIATED CONTENT

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestengg.1c00369.

Detailed Methods and figures referred to in the text including aerosol filtration system schematics, DLS and Bradford and SDS-PAGE analysis for protein-labeled nanoparticles, schematic for pseudoparticle production method, DLS for aerosol feed mixtures, pseudovirus aerosol experiment optimization, impinger design and photo, raw pseudovirus titer data, schematic overview of this study, photos of disassembled modules, detailed SEM imaging analysis, nitrogen and mercury porosimetry results, contact angle analysis, annotated pore size analysis SEM images, theoretical air flux prediction results, and long-term filtration flow characteristics (PDF)

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