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Original Article

Polymer Additives to Personal Protective Equipment can Inactivate Pathogens

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Abstract—Face masks have been proven to be medicine's best public health tool for preventing transmission of airborne pathogens. However, in situations with continuous exposure, lower quality and "do-it-yourself" face masks cannot provide adequate protection against pathogens, especially when mishandled. In addition, the use of multiple face masks each day places a strain on personal protective equipment (PPE) supply and is not environmentally sustainable. Therefore, there is a significant clinical and commercial need for a reusable, pathogen-inactivating face mask. Herein, we propose adding quaternary poly(dimethylaminohexadecyl methacrylate), q(PDMAHDM), abbreviated to q(PDM), to existing fabric networks to generate "contact-killing" face masks—effectively turning cotton, polypropylene, and polyester into pathogen resistant materials. It was found that q(PDM)-integrated face masks were able to inactivate both Gram-positive and Gram-negative bacteria in liquid culture and aerosolized droplets. Furthermore, q(PDM) was electrospun into homogeneous polymer fibers, which makes the polymer practical for low-cost, scaled-up production.

Keywords—Quaternary ammonium polymers, Personal protective equipment, Bacterial contact-killing, Aerosolized bacteria, Free radical polymerization.

INTRODUCTION

The COVID-19 pandemic has shown that despite significant advances in healthcare and biotechnology, face masks are still the most impactful public health tool available for preventing transmission of airborne pathogens. Surgical masks have shifted from being a 'healthcare product' to an essential 'consumer product', which triggered personal protective equipment (PPE) shortages across the world.[5] Historically, face masks take only a few cents to manufacture, but due to limited supply and high demand between primary healthcare providers, patients, and everyday people, the price of single-use PPE has risen exponentially, up to $6 per mask in March 2020, over a 400% markup.[3,39]

Even the gold-standard of mask PPE, such as N95 respirators and their international counterparts (e.g., KN95), are only effective for preventing transmission with proper sterile techniques and 'fit tests'; they can only be sterilized up to three times before filtering efficiency decreases.[6] Many consumer mask users also lower the efficacy of face masks by either wearing a mask incorrectly, wearing an improper face masks, or inappropriately reusing face masks without proper decontamination protocols (based on CDC suggestions).[7,11] 3D printing of PPE has also become increasingly popular, however the masks produced in this way are not FDA approved and have issues regarding heterogeneity and inefficient filtration capacity.[10,41] Newer antiseptic face mask materials have claims of integrating nanofibers, nanoparticles, copper and metal particles/fibers, heat-producing components, and surfactants to generate an antimicrobial and antiviral effect; however, these are not yet widely available to consumers due to high costs or issues in reproducibility.[18,19,32,42,45]

Traditional surgical face mask materials—including polypropylene, polyethylene, cotton and polyester—allow for bacterial and viral adhesion.

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While this may be insignificant for single use PPE, for consumers who seek to use these masks daily, non-compliance and mishandling of PPE greatly increases risk of transmission and homemade solutions have been found to only filter 10–60% of respiratory-sized droplets.[27,35] This poses a risk for transmission, especially for those who are less-dexterous and forgetful. While the need for a contact-killing face mask is obvious in the era of COVID-19, a growing geriatric population, rapid urbanization, and the increasing prevalence of airborne diseases are expected to make the demand for reusable face masks continue to increase.[4] Therefore, there is a current patient and consumer need for a low-cost, reusable face mask that prevents respiratory transmission by contact killing viruses and bacteria while maintaining breathability.

Our proposed solution is integrating a polymeric quaternary ammonium compound (polyQAC), specifically quaternary ammonium poly(dimethylaminohexadecyl methacrylate), q(PDM), into existing PPE solutions to contact-kill bacteria and viruses to prevent secondary transmission of diseases from PPE mishandling. Quaternary ammonium compounds (QACs) are a class of cationic polymers typically used in food processing and surface sanitation due to their ability to contact-kill bacteria and viruses while reducing protein adhesion to surfaces.[8] While QACs have been utilized for decades in sanitation applications, polymerized QACS (polyQACs) have recently shown to have potent antibacterial and antiviral properties and limited cytotoxicity, making them attractive for biomedical implants, wound dressings, catheters and dental implants.[12,13,29] Many recent studies regarding these materials cite enhanced therapeutic indices and a lower likelihood of developing antibacterial resistance in comparison to their monomer counterparts.[46]

Herein, we plan to leverage the antibacterial and antiviral activity of polyQACs to augment standard surgical face masks to ensure sterility over time, thereby allowing the reuse of PPE in both a clinical and consumer setting. Current combination products for contact-killing masks rely on metal nanoparticles, heat, and electrical currents; however, all of these solutions require significant changes in the way we manufacture PPE.[15,20,36] On the other hand, q(PDM) can be integrated into existing PPE by either (1) spraying or soaking a q(PDM) solution onto a face mask and allowing it to dry or (2) electrospinning fibers of pure q(PDM), which is a relatively low-cost and high-efficiency technique capable of manufacturing large quantities of fibers in a relatively short period of time.

MATERIALS AND METHODS

Q(PDM) Synthesis

Q(PDM) was synthesized in a two-step reaction (1) a modified Menschutkin reaction to synthesized a quaternary ammonium methacrylate monomer (q(DMAEMA)), which is based off of previously studies[14,44] and (2) free radical polymerization of q(DMAEMA) with AIBN (Fig. S1). Briefly, 1-bromohexadecane (9.18 mL, 30 mmol) and DMAEMA (4.78 mL, 30 mmol) were dissolved in ethanol (EtOH) (9 mL) and reacted at 70 °C for 24 h. under N_2 atmosphere and stirred. The resulting solution was then distilled under vacuum at 60 °C to remove both excess EtOH and residual monomethyl ether hydroquinone (polymerization inhibitor) from the DMAEMA stock.

Once distilled for around 1 h, the solution was heated to 60 °C (stirred) and AIBN (54.2 mg, 0.33 mmol) was added to solution and allowed to react for 24 h. Upon reaction completion, the mixture was observed to be viscous and a transparent yellow color was observed. Synthesized polymer was dried and stored at 2 °C.

NMR and Molecular Weight Estimation

Nuclear magnetic resonance (NMR) was used to verify successful conjugation during synthesis steps. All spectra of presented chemical species were recorded by Bruker 300 MHz NMR system (Bruker, Germany) in DMSO-D_6. ^{1}H-NMR spectra were analyzed on Bruker Topspin software (4.0.9). Unique peak integrals for polymer end groups, backbone, and functionalized alkane groups were used to quantify percent functionalization and molecular weight.

Bacteria-Broth Shake Test

Polymer anti-bacterial properties were assessed with a modified broth-dilution test against both S. *aureus* and *E. coli*. 3 mL of ~ 10^9 colony forming units (CFU)/mL were incubated for 24 h at 37 °C with 40–50 mg of dried q(PDM). Before and after incubation, an area scan of each well was taken at 600 nm on a Synergy H1 Hybrid Multi-Mode Microplate Reader (BioTek Instruments, Inc., Winooski, VT). Results were reported relative to optical density (OD) at $t = 0$ for each group and percent growth reduction normalized to polymer mass.

Q(PDM)-Coated Mask Preparation

7.5–20 w/w% solutions of q(PDM) were made in EtOH by mixing dried polymer with solvent on an end-over-end mixer at room temperature, overnight (ON). Polymer solutions were evenly applied to general surgical face masks using pipettes. Solutions were allowed to dry at room temperature and mask mass difference was used to calculate polymer density (mg polymer/mm^2 mask). Not all of the applied polymer remains on the mask after coating, as flaking and polymer precipitation on the tabletop was observed.

Scanning Electron Microscopy (SEM)

SEM sample images were completed at the Swagelok Center for Surface Analysis of Materials at Case Western Reserve University. Samples were mounted on carbon tape, sputter coated with palladium, and imaged on a FEI Helios NanoLab 650; 10 kV acceleration voltage. Fiber diameters were determined using ImageJ.

Upright-Cup Permeability Test

Material permeability was assessed by an upright cup test, commonly used for fabric material analysis based off of ASTM E96.[1] ~ 8–10 mL of water was weighed out into a glass scintillation vial and face mask materials were secured with parafilm to form a seal. Samples were incubated at 37 °C, 18% humidity, for 24–72 h. and loss in water mass was recorded.

Nebulized Bacteria Filtration Assay

Modified PPE materials were tested *in vitro* for filtering and contact-killing efficiency *via* a modified protocol based on an Andersen cascade impactor assembly. As shown in Fig. 1, a nebulizer was placed in series with a Precision Medical EasyComp Air Compressor and aerosolized droplets were pushed through the sampled material, with flow-through collected on a sterilized petri dish. Two conditions were tested—(1) 'chronic exposure', where bacteria were nebulized without a significant pressure gradient for 10 min and (2) 'acute exposure', where bacteria were nebulized and exposed to the material surface for 30 s with ~ 2 psi pressure gradient, to simulate a 'sneeze' or 'cough'. Cultured bacteria, either S. aureus or E. coli (~ 10^9 CFU/mL), were diluted 1:1 with diH_2O and loaded into the nebulizer cup and replaced for each trial. While we are using a relatively high concentration that may not be representative of physiologic ''sneezing'', previous work has found that concentrations ranging from 10^7 to 10^9 CFU/mL can be used to analyze pathogen inactivation.[17,30,43] The petri dish collecting flow-through (FT) and a swab of the front (F) and the back (B) of the mask was incubated ON at 37 °C. Both 'perfect seals' and 'imperfect seals' (5–10 needle holes poked through the mask before use) were tested. For each trial group (e.g., all 0.1 mg/mm^2 q(PDM) masks), swatches from the same facemasks were used to standardize testing.

Surface Contact-Killing Assay for Face Mask Fabrics

In order to quantify the modified PPE's ability to inhibit bacterial growth on its surface, we used a modified surface contact-killing assay. Briefly, 200 μL of diluted confluent bacteria (1:3 dilution in diH_2O) was applied to the front surface of the face mask and spread. At each recorded timepoint, a sterile scraper was used to sample a small area of the material and applied as a single streak to a petri dish. 24 h after the sample timepoint, the streak was imaged for CFU analysis.

Viral Inactivation Assay

Viricidal activity was performed on treated fabric swatches of according to ASTM standard E3179-18.[2] Briefly, swatches were inoculated with a bacterial/viral load. After 30 s (acute exposure) and 10 min (chronic exposure) the fabric surfaces were cultured. The reduction of colony-forming (CFU) or plaque-forming (PFU) was recorded for bacteria and virus samples, respectively. Model resistant-bacteria (MRSA), lipid-enveloped virus (Phi6), and capsid-encapsulated bacteriophage (MS2) were tested.

Electrospinning q(PDM) Fibers

Electrospinning was performed using a Spraybase® (Cambridge, MA) Electrospinning Starter Kit, a 20 kV power supply and a syringe pump. Briefly, polymer solutions were loaded into a 3 mL syringe and were spun with varied rates (mL/h), voltage potentials (1-20 kV), and solvents (THF, DMF, EtOH). During this study, electrospinning took place behind a glass shield at standard room temperature (21 °C) and humidity (~ 35–45%). Emitted samples were collected on aluminum foil sheets.

Statistical Analysis

Experiments were all carried out in triplicates, unless otherwise states. Data were expressed as the mean ± standard error. Statistical tests were performed using Student's t test or analysis of variance with p values.

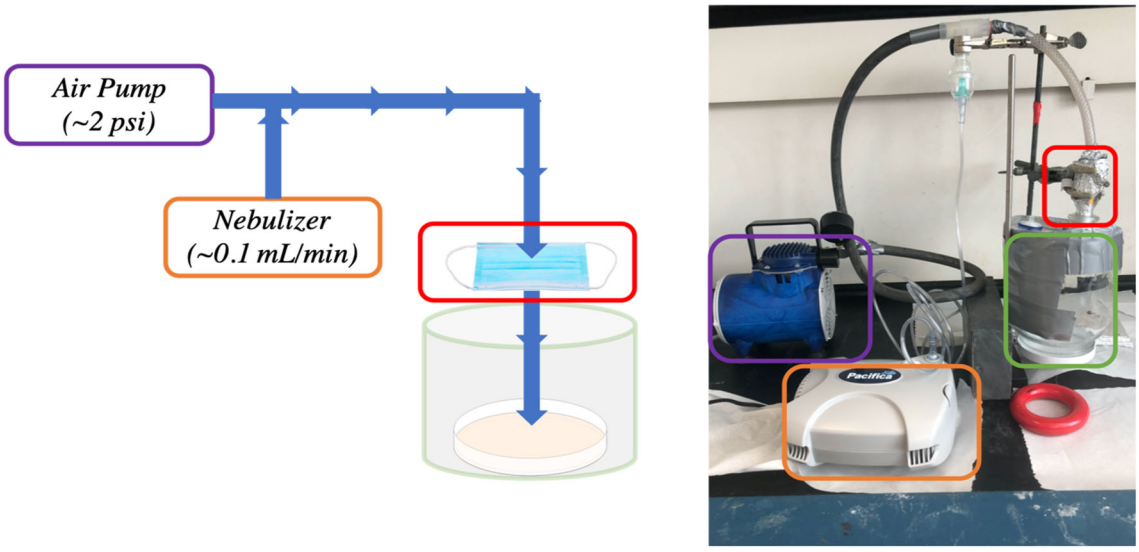

FIGURE 1. Experimental setup for modified PPE filtering and contact-killing efficiency *in vitro*. In future work, the petri dish can be replaced by an Andersen cascade impactor for more detailed particle flow-through data.

RESULTS AND DISCUSSIONS

Q(PDM) Two-Step Synthesis

Q(PDM) synthesis was confirmed *via* ^{1}H-NMR. Confirmation of both free radical polymerization and the successful conjugation of 1-bromohexadecane to DMAEMA backbone was observed by the coexistence of DMAEMA's hydrocarbon (–CH_2–) peaks at δ = 4.52 ppm, hexadecane's terminal methyl group (–CH_3) at δ = 1.37 ppm, and AIBN's two terminal methyl groups (–CH_3) on the ends of each terminated polymer chain at δ = 0.88 ppm. Percent quaternization was determined by comparing peak integrals of hexadecane terminal groups (δ = 1.37 ppm; 3 hydrogens) and DMAEMA's unique '–CH_2–' group (δ = 4.52 ppm; 2 hydrogens), yielding approximately 70% quaternization (Fig. 2). Approximate molecular weight was obtained by comparing peak integrals of AIBN's terminal methyl groups (6 hydrogens per end-group) with DMAEMA's '–CH_2–' group to obtain N number of DMAEMA monomers and N' number of quaternized DMAEMA monomers. Molecular weight was estimated using Eq. 1, derived from molecular weights of monomer, ammonium side chains, and end-groups, respectively.[23]

$$M_w = (N \times 173\ \text{g/mol}) + (N' \times 382\ \text{g/mol}) + (2 \times 68\ \text{g/mol}) \quad (1)$$

Q(PDM)'s molecular weight ranged between 9800–30,000 g/mol [n = 4 batches].

Solid q(PDM) Contact Kills Both Gram-Negative and Gram-Positive Bacteria

To test the contact-killing bulk properties of q(PDM), a simple, bacteria-broth shake test was used, wherein the population of viable bacteria greatly outnumbered the total surface area of the polymer. We found that q(PDM) effectively reduced the growth of both *S. aureus* and *E. coli* (Fig. 3a); however, the polymer was found to be more effective against *S. aureus* than *E. coli*, which matched previous studies with similar polymers.[9] Q(PDM)'s antimicrobial activity also appeared to be surface-area dependent (Fig. 3b) where bacteria were only observed growing in regions without direct polymer exposure. We also confirmed the general mechanism of q(PDM)'s surface-contact inactivation of bacteria (Fig. 3c) as surviving bacteria were only observed on the periphery of the 6-well plates, suggesting that direct contact of q(PDM) to the bacteria is necessary for inactivation.

Q(PDM) can be Integrated into Fabric by Soaking with Volatile Organic Solvents

EtOH solutions of q(PDM) were applied to general surgical face masks and allowed to dry for at least 6 h. at room temperature. 52% (SD 15%) of q(PDM) that was applied was integrated into the fabric of the mask. Polymer integration density (mg polymer/mm^2 mask) was reported for each subsequent experiment and ranged between 0.01 and 0.12 mg/mm^2.

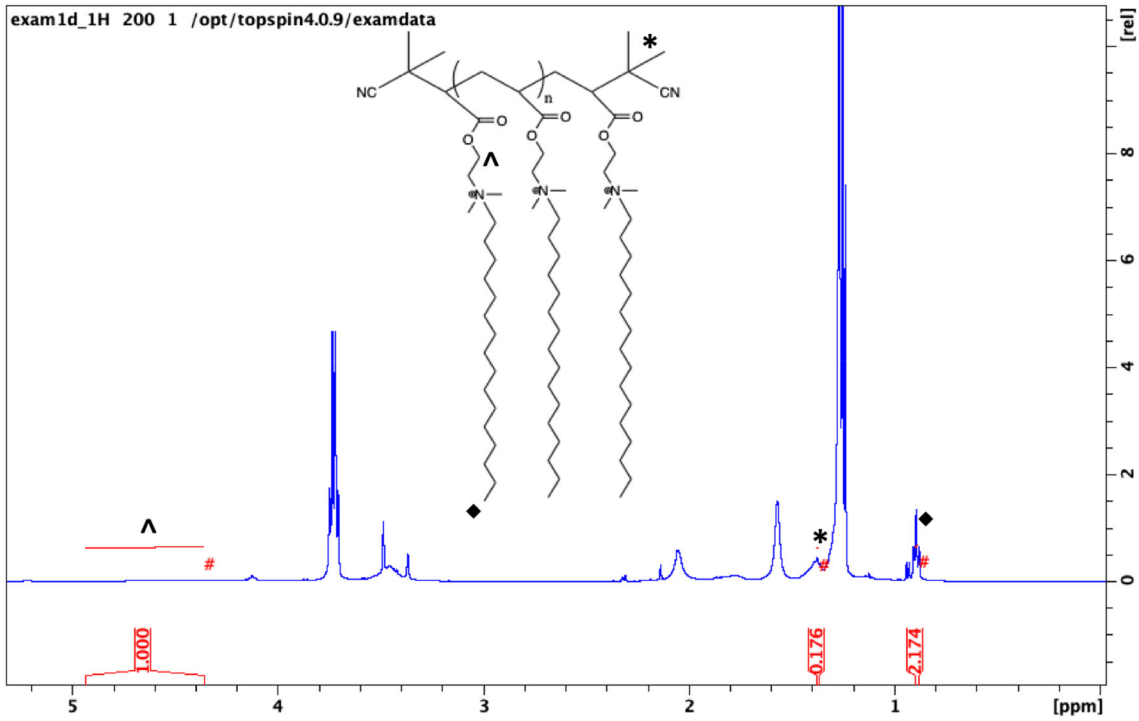

FIGURE 2. ^{1}H-NMR (DMSO-D_6) of q(PDM), with unique peaks at δ = 1.37 ppm (CH_3 hexadecyl end group, ◆), δ = 0.88 ppm (AIBN terminal CH_3, *), and δ = 4.52 ppm (CH_2 neighboring DMAEMA's ester group, ^).

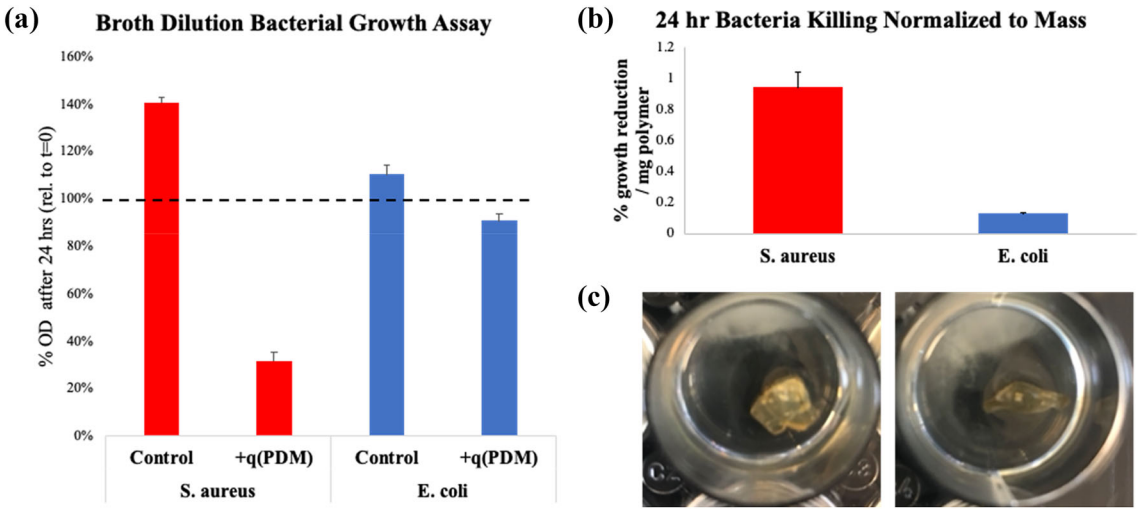

FIGURE 3. (a) Growth reduction (determined by optical density) of *S. aureus* and *E. coli* after 24 h incubation with ~ 40–50 mg solid q(PDM) polymer, compared to a positive control (bacteria-only, no q(PDM)). 100% corresponds to absorbance at t = 0 for each group. (b) Percent growth inhibition normalized to polymer mass for both groups. In all groups (n = 3), error bars are representative of the standard error of the mean. (c) Visual representation (top view of 6-well plate) of q(PDM) in presence of bacteria during broth dilution test. Surviving bacterial colonies were observed on the periphery of the 6-well plates.

PPE permeability after q(PDM) integration was also examined to ensure that the addition of polymer does not impact the breathability or permeability of the existing face mask. Upright cup tests revealed that exposure of EtOH inherently damaged the integrity of the face mask; however, at densities above 0.02 mg/mm^2 permeability was decreased below unmodified face mask controls (Fig. S2). Subjectively, the decreased permeability of the face mask material did not significantly hinder breathability.

SEM images were obtained to ensure that the integrated q(PDM) was evenly distributed among the

existing face mask fibers and showed that an increase in polymer integration yielded an increase in average fiber diameter size (Fig. 4). At higher concentrations (> 0.1 mg/mm^2), droplets of solidified polymer were observed (Fig. S3). Overall, q(PDM) integration with existing fibers was confirmed, increasing existing fiber diameters by 7 μm (0.02 mg/mm^2) up to over 40 μm (0.12 mg/mm^2) (Fig. 4).

Face Masks with q(PDM) can Prevent E. coli and S. aureus Infiltration, even with Imperfect Fitting

Once the polymer was confirmed to have antimicrobial properties, aerosolized bacteria and low-concentration bacteria contact-killing assays were used to better simulate face mask contamination conditions *in vitro* (i.e., droplet exposure, 'sneezes'). 'Chronic exposure' (10 min, nebulizer mist, ~ 5×10^8 CFU/mL) trials showed that despite common belief, general surgical face masks are permeable to bacteria over long-term exposure. Figures 5 and 6 show that upon integration of 0.01 mg/mm^2 q(PDM), bacterial filtration decreases, most likely due to the increased permeability caused by EtOH cracking of existing face mask fiber networks. However, at higher concentrations (> 0.05 mg/mm^2) both live *E. coli* and *S. aureus* were either inactivated or filtered as shown on the (FT) dish, and colonies were unable to be cultured from the back (B) of each mask (Figs. 5 and 6). As the integration density was increased to 0.12 mg/mm^2, nebulized bacteria were significantly—or completely—eliminated from FT and B in both 'acute' and 'chronic' conditions. It was also observed that higher concentration q(PDM) face masks were less likely to absorb condensate than traditional face masks, most likely due to an increase in surface hydrophobicity. Overall, Figs. 5 and 6 qualitatively show that q(PDM)-treated facemasks exhibit noticeable antimicrobial activity against both *E. coli* and *S. aureus*.

Q(PDM) Inhibits Bacteria Attachment and Growth on Face Mask Fabrics

Although bacterial survival on surfaces has been widely reported for both *S. aureus* (> 7 days) and *E.coli* (> 5 days),[22,25,26] we aimed to observe if both Gram-positive and Gram-negative bacteria can remain viable on a surgical face mask over the span of 16 h after contamination, which we predicted would be the intra-day time frame for exposure from handling a contaminated face mask. In both Gram-positive and Gram-negative trails, we observed that viable bacteria survived on the control and EtOH face masks for up to 16 h, however *E. coli* was unable to survive over 1 h on face masks with > 0.04 mg/mm^2 integrated polymer (Fig. 7a) and *S. aureus* after 16 h (Fig. 7b). Furthermore, at high polymer densities (0.12 mg/mm^2) *E. coli* as unable to be detected directly after contact ($t = 0$ h) and *S. aureus*, although more resilient, was not detected after 1 h on a 0.12 mg/mm^2 q(PDM) surface.

Model Resistant Bacteria and Enveloped Viruses are Inactivated After 30 s of Polymer Exposure

As a preliminary test against other pathogens like viruses, we exposed treated masks to a model resistant-bacteria (MRSA), lipid-enveloped virus (Phi6), and capsid-encapsulated bacteriophage (MS2) (Fig. 8). Based on our previous studies, only 0.1 and 0.12 mg/

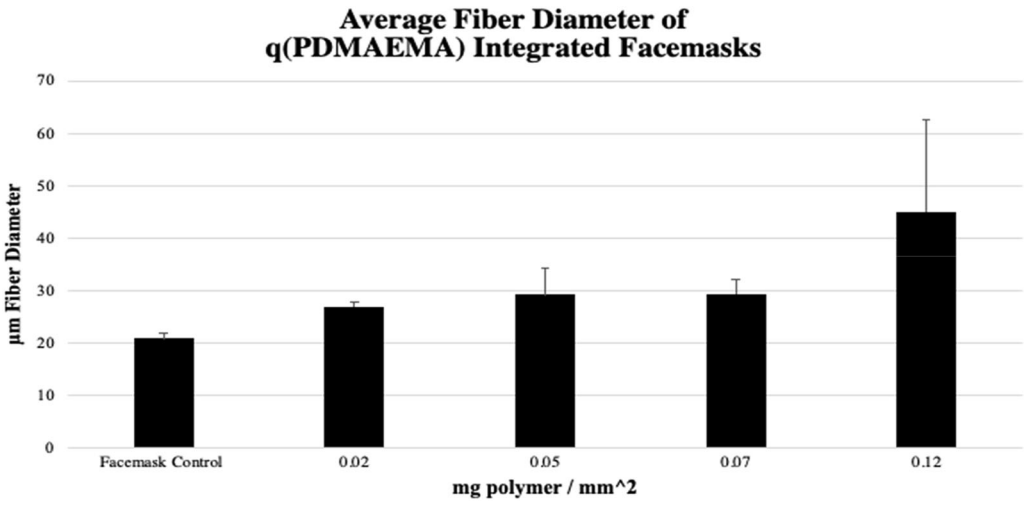

FIGURE 4. SEM image obtained average fiber diameter sizes of q(PDM) integrated face masks ($n = 2$). An average of 10 fiber diameters were measured per image (~ 20–30 total per group). All groups were significantly different from control face masks ($p < 0.05$)

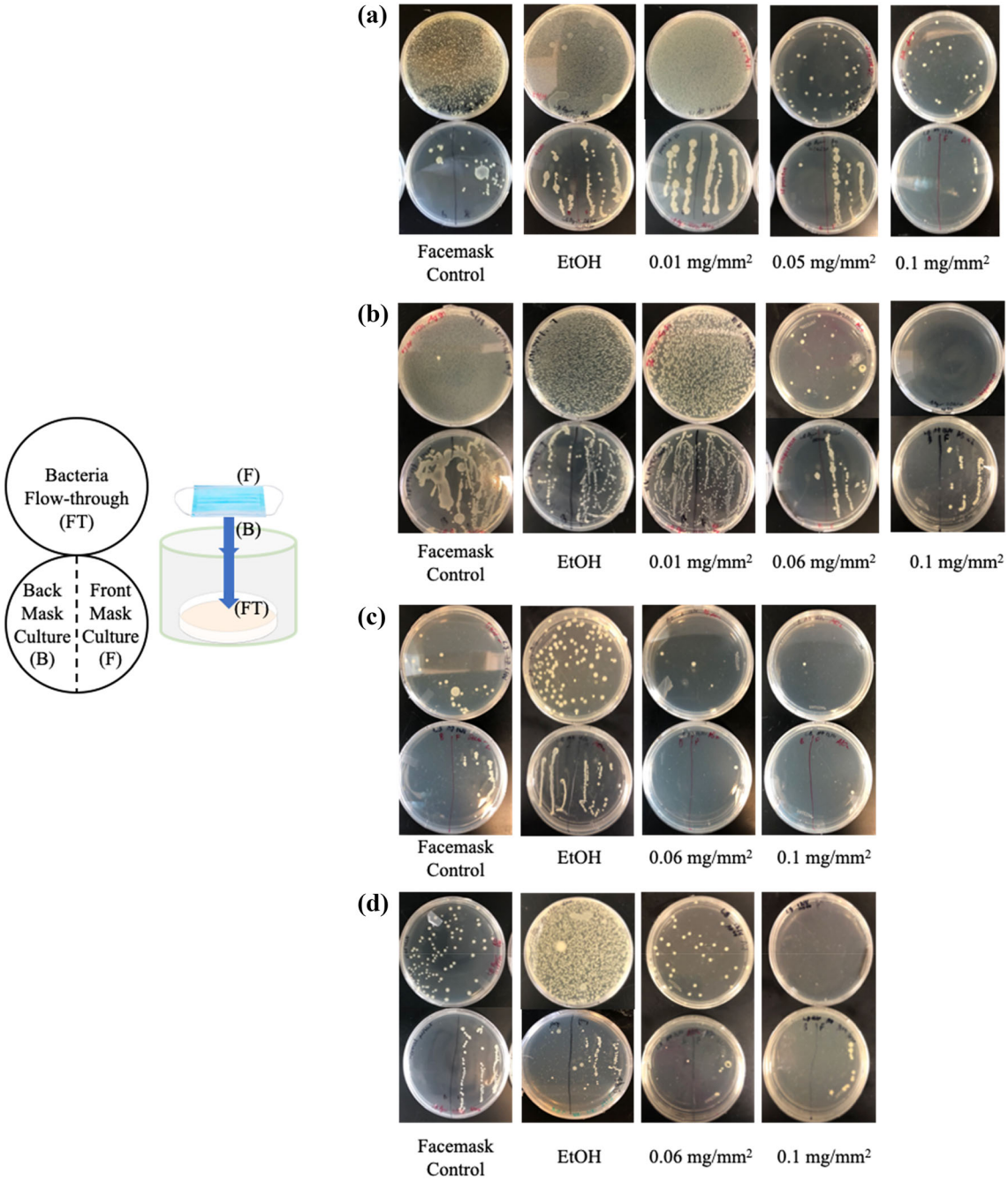

FIGURE 5. Nebulized E. *coli* 'chronic exposure' face mask test for (a) 'perfectly sealed' face masks and b) 'imperfectly sealed' face masks (~ 5 holes). 'Acute exposure' (e.g., sneeze), (c) 'perfectly sealed' face masks and (d) 'imperfectly sealed' face masks.

mm^2 were trialed based on their high activity against both *E. coli* and *S. aureus,* however due to q(PDM)'s lower efficacy against MS2, we also tested 0.22 mg/mm^2 for the capsid-enveloped group.

Q(PDM) can be Electrospun into Micro-Scale Fibers

Exploring alternative methods of integrating q(PDM) into existing fabrics and PPE, we investigated whether the polymer was able to be electrospun to produce homogenous, polymer fiber mats. Electrospinning, and newer methods including melt electrospinning,[24] are scalable manufacturing processes used to efficiently produce micro- and nanoscale fiber networks from polymers.[40] Q(PDM) was dissolved in a variety of organic solvents (EtOH, DMF, THF) and at different weight percentages (5%, 10%, 15%). It was observed that EtOH was able to repeatedly dissolve

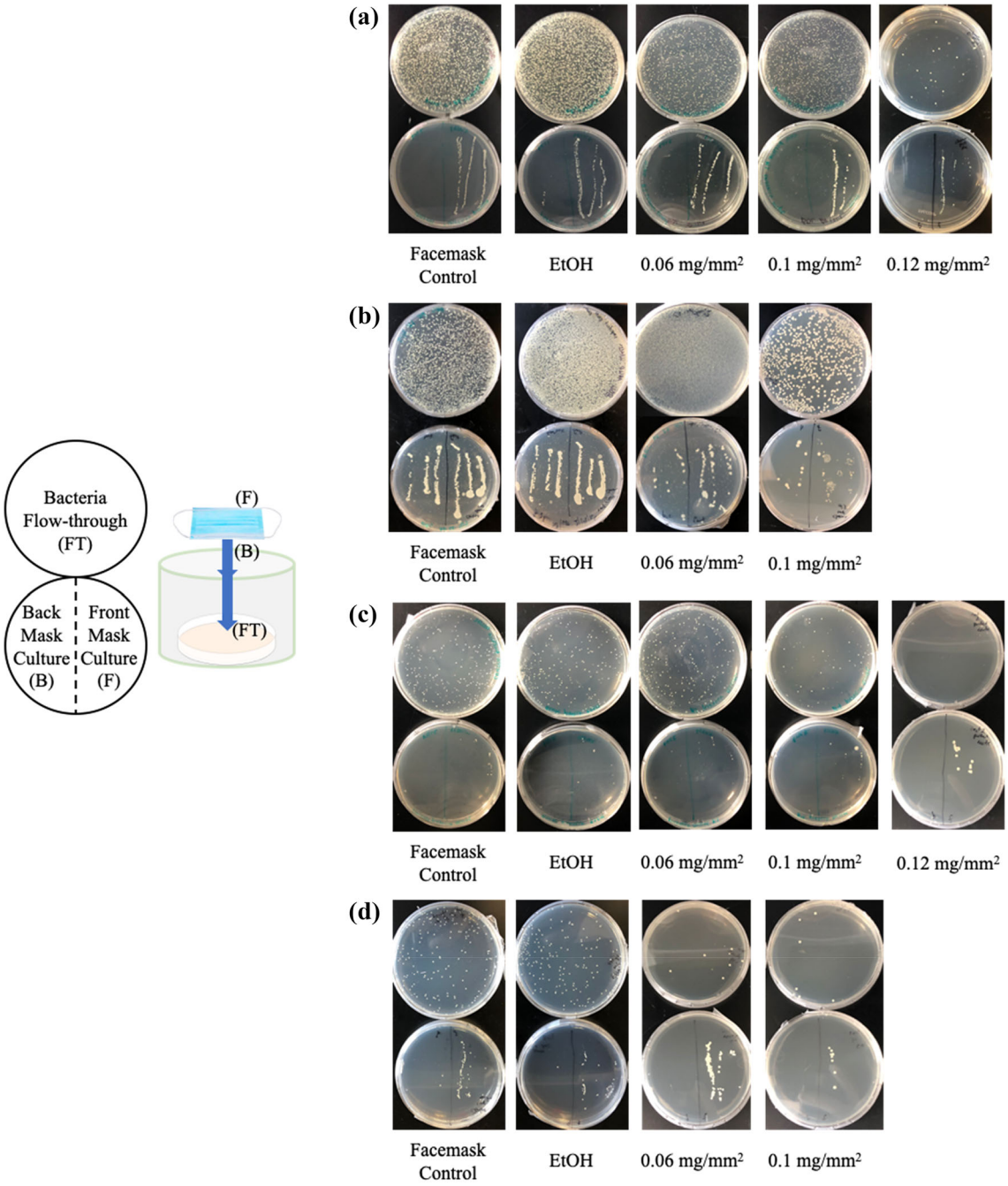

FIGURE 6. Nebulized *S. aureus* 'chronic exposure' face mask test for (a) 'perfectly sealed' face masks and (b) 'imperfectly sealed' face masks (~ 5 holes). 'Acute exposure' (e.g., sneeze), (c) 'perfectly sealed' face masks and (d) 'imperfectly sealed' face masks.

q(PDM) at room temperature and was able to be fed through the electrospinning tubing without significant clotting (both DMF and THF were also compatible solvents, however more frequent clotting was observed). Electrospinning parameters, including solution flow rate and voltage potential, were varied, and it was found that ~ 10% w/w EtOH solution at 1 mL/h between 8 and 10 kV produced visible, white microfibers between 20 and 80 μm in diameter (Table 1, Fig. S5). 5% w/w was also able to generate fibers, however light microscopy images revealed that the fibers were heterogeneously combined with polymer droplets, suggesting electrospraying was also occurring (Fig. S4). 15% w/w caused clotting at the emitter and was unable to produce fibers. It should be noted that optimal parameters for electrospinning q(PDM) were

dependent on batch and environmental conditions (e.g., temperature/humidity) and was adjusted accordingly until fiber formation was observed.

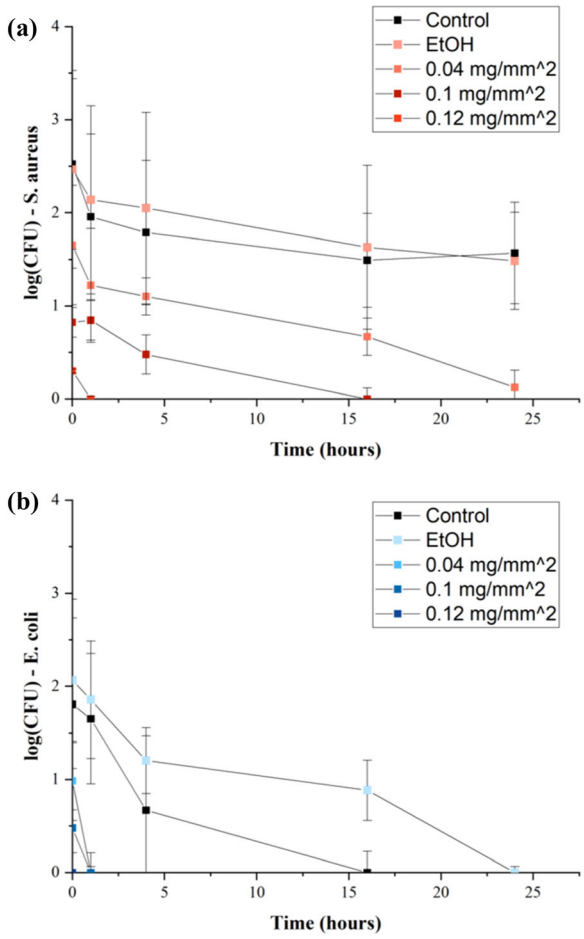

FIGURE 7. Surface contact-killing assay for both (a) *S. aureus* and (b) *E. coli*. Error bars are representative of standard error of $n = 3$ trials. Control and EtOH groups were found not to be statistically significantly ($p = 0.739$), while all modified surfaces were significantly lower than controls ($p < 0.05$).

DISCUSSION

Herein, we outline the synthesis, basic characterization and efficacy of a methacrylate-based quaternary ammonium polymer, abbreviated q(PDM), against model bacteria *S. aureus* and *E. coli*. along with resistant-bacteria (MRSA), lipid-enveloped virus (Phi6), and capsid-encapsulated bacteriophage (MS2).

Q(PDM) synthesis was found to be highly dependent on order of quaternization vs. polymerization, and could potentially be altered in future studies varying molar ratios, solvent amount and time of reactions. Previous synthesis attempts suffered from either insufficient quaternization (polymerize DMAEMA, then quaternized; ~ 13 kg/mol, ~ 16% quaternization) or insufficient polymerization ('one-pot' synthesis; ~ 8 kg/mol, 24% quaternization). The reported synthesis of first quaternizing the active branches then sequentially polymerizing functionalized monomers yielded the highest percentage of functional groups (~ 70%) along with sufficient polymer chain size (Fig. 2).

When integrating q(PDM) with our face mask material, we found that the administration of pure anhydrous EtOH dehydrated the fibers, causing increased permeability (Fig. S2) which later translated to a lower filtration efficiency (Figs. 5 and 6). However, at higher integration densities, these 'cracked', dehydrated fibers are filled with q(PDM) upon solvent evaporation (Figs. 4 and S1). While integration of q(PDM) to the facemask was confirmed, further analysis of the characterization of this integration will be necessary in future studies.

To help mitigate these concerns, we found that generating homogenous fiber mats (Table 1, Fig. S5) using electrospinning might be a scalable manufacturing process to replace manual application of the polymer onto an existing surface. Based on results in Table 1, narrower concentration ranges of q(PDM) were attempted, and it was wound that 7.5% w/w EtOH solutions produced fibers and prevented clotting

TABLE 1. Electrospinning of q(PDM) with varied concentrations and electrospinning parameters.

Percent weight polymer in EtOH	Emitter flow rate (mL/h)	Voltage (kV)	Distance from collector (cm)	Observation
5% w/w	0.6	0–15	20	No fiber formation
	1	14	20	Fiber formation with droplets
	1.5	11	20	Coating and small fiber formation
10% w/w	0.6	0–15	20	No fiber formation
	1	8–10	20	Extensive fiber formation
	1.5	10	20	Extensive fiber formation, larger diameter
15% w/w	–	–	–	No fiber formation (too viscous)

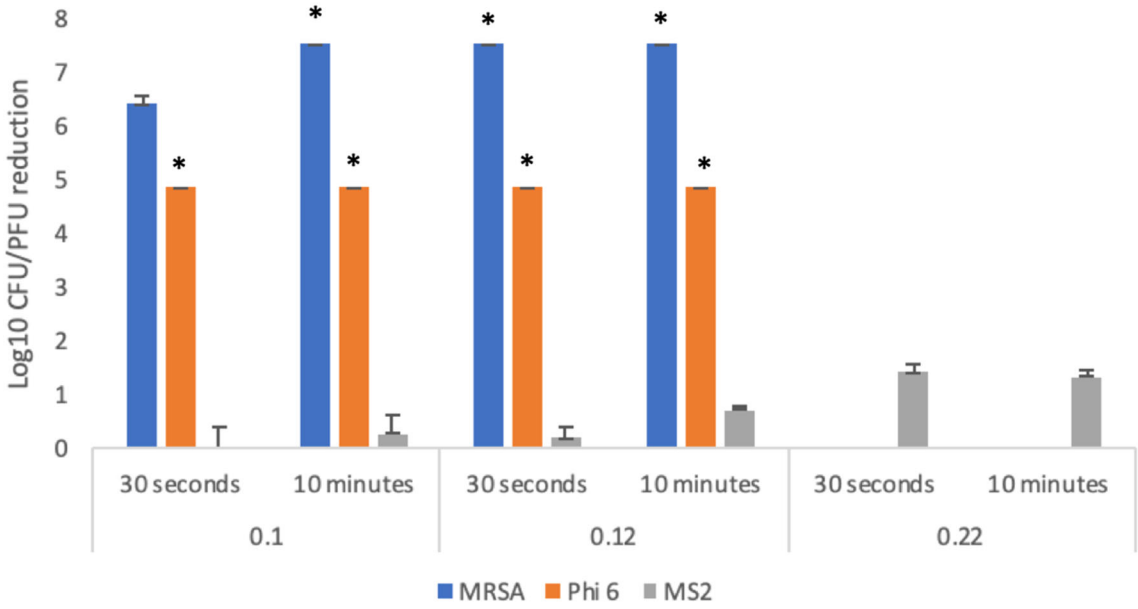

FIGURE 8. Pathogen inactivation efficacy assay of q(PDM)-integrated face masks (0.1, 0.12 and 0.22 mg/mm^2) against MRSA, Phi6 and MS2 with exposure times of 30 s and 10 min based on ASTM standard E3179-18. *Groups in which exposed pathogen was completely inactivated. Log10 reductions were compared to soiled, untreated mask swatches. Error bars are representative of the standard error (n = 3).

in the electrospinner emitter. 7.5% w/w produced q(PDM) samples were then imaged *via* SEM and was observed to have fiber diameters ranging from 100 nm to 100 μm, with a notable 'braided' structure (Fig. S5). However, futures studies must validate and characterize the mechanical strength and efficacy of such electrospun fiber mats. In addition, the impact of applying a high electrical charge to a QAC polymer may induce polymer chain degradation resulting in alterations in physical and mechanical properties, which must be investigated in future studies if electrospinning is to be considered a potential manufacturing method.[31]

Face masks treated with q(PDM) were shown to have antimicrobial properties towards both *S. aureus* and *E. coli*. in aqueous (Fig. 3), aerosolized (Figs. 5 and 6), and solid (Fig. 7) conditions. Differences in bactericidal activity between broth-dilution tests (Fig. 3) and the aerosolized/surface-contact bacteria assay (Figs. 5, 6, and 7) are most likely attributed to differences in *E. coli* and *S. aureus* cellular structures: Gram-positive *S. aureus* have significantly thicker peptidoglycan cell walls (~ 50 nm) compared to Gram-negative *E. coli's* inner/outer lipid membranes (~ 2 nm).[28] Since quaternary ammonium polymers act by disrupting bacterial membranes, we believe that while *E. coli* can survive better as a biofilm, as observed in the broth-dilution assay, they are more easily killed in aerosolized-form or low-concentration solutions. Conversely, *S. aureus* is able to survive more effectively in aerosolized form and at low-concentrations as its peptidoglycan cell walls are more resistant towards q(PDM)'s active moieties, and therefore requires higher polymer concentrations to effectively inactivate. It was also noted that EtOH-treated masks performed more poorly than untreated facemasks, which we hypothesize is also due to a loss of permeability from solvent administration.

q(PDM)-treated masks inactivated MRSA and Phi6 similarly to *E. coli* and *S. aureus* after 10 min of exposure at all polymer concentrations, which supports the proposed membrane-disrupting mechanism of our QAC (Fig. 8). However, we found that MS2 was less effectively deactivated at lower concentrations of q(PDM) and, even at the highest q(PDM) concentration of 0.22 mg/mm^2, yielded less than a 2-log reduction. We attribute these differences in pathogenic activity to the structural differences of Phi6 and MS2: Phi6 is a double-stranded RNA virus with a unique lipid membrane around their nucleocapsid while MS2 is a positive-sense single-stranded RNA virus with a capsid. We believed this reduced efficacy is the result of MS2's protein-capsid structure, which aligns with previous studies citing higher survival rates for capsid-encapsulated viruses when exposed to QACs, likely because capsids are less prone to disruption by the QACs long alkane chain.[38] Regardless, as Phi6 is typically considered an acceptable surrogate for coronaviruses like SARS-CoV-2, we believe this data supports that q(PDM) is sufficiently effective to be used in

applications where bacteria and enveloped-viruses are most common, which includes many respiratory infections.[16] While the pathogen inactivation properties of q(PDM) were characterized in this study, further work will need to be done to characterize the biocompatibility of q(PDM) with the skin and lungs if polymer fibers were to flake and be inhaled. Understanding and quantifying our additive's impact on overall transmissibility of pathogens (e.g., with COVID ferret models) and the potential health risks with misuse of material failure will be the focus on future work.[37] Also, the environmental impact of QACs are currently not well understood and environmental implications of q(PDM) would also require further investigation.[21]

To our knowledge, this is the first integration of q(PDM) for the purpose of generating antimicrobial fabrics and personal protective equipment. Herein, we outlined a method to synthesize a quaternary ammonium polymer, q(PDM), coated existing PPE fabric fibers with q(PDM) and generated microfibers of pure q(PDM) *via* electrospinning. While previous groups have investigated the antimicrobial properties of similar polymers, this is the first study repurposing the polymer to be integrated into fibrous textile networks.[33,34] q(PDM) was observed to be effective against Gram-positive and Gram-negative bacteria in both high-concentration broths and low-concentration nebulized droplets. The modified surfaces also yielded significantly decrease survival time of bacteria on its surface. This study presents q(PDM) as a potential solution for generating antiseptic personal protective equipment at a relatively low cost (manufactured at an estimated ~ $0.50 per gram polymer at benchtop scale). While $0.50 is likely near or above current industry costs for producing surgical facemasks at-scale, we believe that treating a used mask might be an attractive alternative to disposing and repurchasing masks for consumers. In addition, the outlined chemical synthesis for q(PDM) only requires ethanol as a reaction solvent, which further contributes to ease of scalability at an industrial scale since a single batch reactor can be used in the future, however optimizing and scaling q(PDM) will require further investigation. In addition, further characterization of q(PDM) attachment to face mask fibers is needed, specifically to ensure that delamination caused by mechanical forces from repeating breathing cycles does not occur. Furthermore, based on q(PDM)'s mechanism of action of membrane disruption, potential viricidal activity makes the material a promising additive to PPE and air-filters to counter airborne pathogens, including viruses like COVID-19.

SUPPLEMENTARY INFORMATION

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CONFLICT OF INTEREST

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ETHICAL APPROVAL

All authors listed have agreed to be named as authors on this manuscript. All external work has been properly acknowledged and this manuscript represents original, and not previously published, work. All data is true and accurate to the knowledge of the authors.

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