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Violacein-embedded nanofiber filters with antiviral and antibacterial activities

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ARTICLE INFO

Keywords:
Violacein
Nanofiber filter
Antiviral activity
Antibacterial activity
Personal protective equipment
Self-sterilization

ABSTRACT

Most respiratory masks are made of fabrics, which only capture the infectious virus carriers into the matrix. However, these contagious viruses stay active for a long duration (~7 days) within the fabric matrix possibly inducing post-contact transmissions. Moreover, conventional masks are vulnerable to bacterial growth with prolonged exposure to exhaled breaths. Herein, we combined violacein, a naturally-occurring antimicrobial agent, with porous nanofiber membranes to develop a series of functional filters that autonomously sterilizes viruses and bacteria. The violacein-embedded membrane inactivates viruses within 4 h (99.532 % reduction for influenza and 99.999 % for human coronavirus) and bacteria within 2 h (75.5 % reduction). Besides, its nanofiber structure physically filters out the nanoscale (<0.8 μm) and micron-scale (0.8 μm – 3 μm) particulates, providing high filtration efficiencies (99.7 % and 100 % for PM 1.0 and PM 10, respectively) with long-term stability (for 25 days). In addition, violacein provides additional UV-resistant property, which protects the skin from sunlight. The violacein-embedded membrane not only proved the sterile efficacy of microbe extracted pigments for biomedical products but also provided insights to advance the personal protective equipment (PPE) to fight against contagious pathogens.

1. Introduction

In the 21st century, with formidable industrial capacities and widely accessible public health systems, the global pandemic of coronavirus disease 2019 (COVID-19) leaves a huge impact on the global community and our livelihoods [1]. One of the most noticeable changes to our day-to-day routine is the mandated usage of masks in public and/or indoor spaces [2]. Since COVID-19 transmits through droplets and aerosols, personal protective equipment (PPE) such as respiratory masks serve as the most immediate and effective solution to protect ourselves from airborne virus carriers discharged from infected persons [3,4]. Commercial respiratory masks are typically made of inexpensive melt-blown polymer filters, which rely on an electrostatic filtration mechanism to attract and trap the viral sources (droplets and aerosols) and particulates within the filter matrix [5]. However, the reliability of melt-blown (MB) filters is limited by their high vulnerability to external environmental factors such as humidity, which dramatically drops their filtration efficiency over time [6,7]. This is because MB filters rely on an electrostatic filtration mechanism, in which particulates are electrostatically attracted to each of the MB fibers that are poled to high voltages during initial production. Moreover, these filters can easily be contaminated by various pathogens [8]. For example, it has been reported that viruses can survive in a general surgical mask for more than 4 days within the inner layer and 7 days in the outer layer [9], raising concerns for potential risks of secondary transmission through contact with contaminated respiratory masks. Bacterial pathogens have also been reported to survive for 1–90 days on textile materials that are made of cotton, poly/cotton, and polypropylene [5,10], which may lead to adverse health effects upon prolonged use of respiratory masks, especially considering that continued exposure to exhaled breath creates a humid environment

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https://doi.org/10.1016/j.cej.2022.136460
Received 1 March 2022; Received in revised form 11 April 2022; Accepted 14 April 2022
Available online 19 April 2022
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that promotes bacterial growth [10]. Most commercial masks attempt to avoid these issues by designing them to be single-use only, which ends up producing a significant amount of plastic waste to the environment [11,12]. Although vaccines and cures are being deployed even as of now, respiratory masks are still recommended by World Health Organization (WHO) due to the unknown risks associated with variant strains of SARS-CoV-2, which would keep demand for mandatory usage of masks [13,14]. Therefore, there is a clear and present need to design a more sustainable solution to fundamentally address the aforementioned issues with respiratory masks.

One approach to mitigate the issues with pathogen contamination is to directly apply antiviral and/or antimicrobial agents (e.g., polyanionic/cationic polymers, metal–organic frameworks, graphene oxides, and metal particles) onto the filter matrix, either on the outer surface and/or in the interior matrix, to inactivate the captured viruses as well as bacteria [5,15–21]. However, previous studies often accompanied several problems such as poor adhesion, nonuniform coverage, and heavyweight of antimicrobial agents, making them impractical [22,23]. Also, the application of these compounds could alter or damage the surface property of fibers, deteriorating the electrostatic properties and filtration efficiency.

Most importantly, the non-toxicity and biocompatibility of these agents should be guaranteed if they are to be used in any consumer product that can directly influence the user’s health. In this sense, violacein is a naturally occurring antimicrobial substance that is cultured from a particular bacterial strain (e.g., C. violaceum) [24,25]. Studies have confirmed that violacein exhibits lower toxicity and better biocompatibility than synthetic antibiotic agents [24,26,27]. Their sustainable microbe-based production method and relative safeness for the human body ensure that violacin-based personal protective equipment can be very effective while also commercially viable. The antimicrobial activity of violacein can be explained by i) the interruption of cell membrane induced by pyrrolic N–H moieties in violacein coupled with the permeabilization of violacein into the membrane giving rise to an efflux of integral cellular components (e.g., ions, ATP, and proteins) and ii) the subsequent disruption of osmotic balance in intracellular systems resulting in the attenuation of cell growth and further cell death [25–28]. For these reasons, violacein has long been used for medical and clinical applications by humankind throughout history [25,29]. However, as of now, violacein has usually been utilized in combination with hydrophilic scaffolds (e.g., polyvinyl-alcohol (PVA), polyvinyl-pyrrolidone (PVP), and nylon-66) through dyeing/dip-coating methods [30–32], which lack resistance to aerosols/droplets and are highly susceptible to humid conditions, limiting their usage for face mask applications. Moreover, considering the low solubility of violacein in water [33], hydrophilic polymer scaffold would not be suitable in terms of compatibility and affinity with violacein.

Herein, we propose a strategy to fabricate nanofiber membranes that incorporate violacein (VIO) as a highly efficient respiratory face mask filter that is capable of sterilizing itself (Fig. 1A). The VIO compound was directly mixed with hydrophobic polyacrylonitrile (PAN) polymer-based solution to be electrospun into nanofibers with a uniform embedment of VIO, a bottom-up approach that is fundamentally different from typical dyeing/dip-coating methods. In particular, the polyacrylonitrile (PAN) with liquid and aerosol resistances acts as an effective barrier for pathogen viral/bacterial ingress [34]. Moreover, the electrospun nanofiber network with fiber diameter under 1 μm - that is far thinner than the micron-sized fibers in melt-blown filters - exhibits a physical filtration mechanism in addition to the electrostatic filtration. These effects enable PAN nanofiber membrane to retain high filtration efficiencies.
against air particulates (87.8 %, 99.7 %, and 100 % for PM 0.3, PM 1.0 and PM 10, respectively) and viral transmission of droplets and aerosols. Especially, we not only confirmed the antibacterial effects of VIO, but also revealed its antiviral effect on the contemporary strains of contagious viruses (influenza and human coronavirus) and bacteria (Staphylococcus aureus). Taken altogether, in this work, we successfully demonstrate the fabrication of highly-functional porous filter membranes with the uniform embedding of VIO pigments in the interior and exterior of individual nanofibers. The electrospinning of violacein-containing nanofiber membrane can be directly adapted to conventional electrospinning operations established in the industry for immediate mass production. The antiviral, antibacterial, and UV-protection effects of VIO-containing nanofibers are discussed, considering their implications for the production of practical filter membranes. Also, the experimental methods developed in this study (e.g., particle observation integrated with laser-beam, antiviral test on filter materials) are anticipated to serve as a useful guideline for the evaluation of bioprotective filter materials in general.

2. Experimental Section

2.1. Materials

Polyacrylonitrile (PAN) (Sigma-Aldrich, Typical grade) with a molecular weight (Mw) of 150,000 g mol⁻¹ and N, N-Dimethylformamide (DMF) (Daejung, 99.5%) were used as received. Violacein was supplied from CJ Cheiljedang White BIO technology.

2.2. Characterisation

Fourier transform infrared spectroscopy (FT-IR) analysis was recorded on Shimadzu IRTracer-100 with ATR mode. SEM images were attained with Philips XL30S with beam energies of 10 kV. TEM observations were performed using Cs-Corrected Scanning Transmission Electron Micros produced from JEOL (JEM-ARM200F) at 200 kV. The UV absorption spectra were collected at wavelengths in the range of 280–400 nm using UV/Vis/NIR Spectrophotometer by Shimadzu (SolidSpec-3700). The stress–strain curve was attained using a universal testing machine supplied from Shimadzu (Japan, AGS-X-STD) with a crosshead speed of 2.5 mm min⁻¹ for the specimen with a length of 25 mm, a width of 25 mm, and a thickness of 20 μm.

2.3. Synthesis of VIO-NF and PAN-NF

The series of VIO-NFs were prepared by electrospinning a polymer solution containing PAN, Violacein (VIO), and DMF. In typical, the polymer solution with the weight ratio of PAN and DMF of 1:9 was agitated at 200 rpm with a magnetic stirrer for 12 h at 70 °C. After completely cooling down the agitated polymer solution to room temperature, 1 and 2 g of VIO were added in the solution to prepare VIO-NF with lower content (VIO-NF-L) and VIO-NF, respectively. For the control sample of PAN-NF, electrospinning solution (PAN:DMF = 1:9 (weight ratio) without incorporating VIO was used. The electrospinning process was performed at a feeding rate of 10 μL min⁻¹ with a constant voltage of 12 kV for 3 h using a syringe connected to a 21G needle on a stainless still covered current collector. The distance between the syringe needle and the collector was set to 40 cm. Electrospinning for 3 h gives VIO-NF and PAN-NF with 40 μm thickness, while 1.5 h gives 20 μm thickness.

2.4. Synthesis of violacein-containing film (VIO-F)

Violacein-containing film (VIO-F) was prepared by spin-coating of a polymer solution (PAN: VIO: DMF = 1:2:9 (weight ratio)) at 300 rpm on the slide glass (Superior Marienfol, Ground edge, 1 mm thickness). The slide glass covered by polymer solution was vacuum dried at room temperature for 24 h and then the film was detached gently.

2.5. Disc-diffusion antibiotic susceptibility test

One colony (S. aureus) extracted from strains cultured in Mueller-Hinton broth was incubated for 16 – 18 h and then diluted to be McFarland standard 0.5 using 0.9 % NaCl solution. The diluted bacterial solution of 25 mL was spread on Mueller-Hinton agar, and the test sample which was pre-cut into Φ11.8 mm disk diameter put on the center of the agar. All tests were repeated three times for 24 h at 35 ± 2 °C temperature. Afterward, the zone edge was determined at the point of complete inhibition as judged by the naked eye, with the plate held about 30 cm from the eye. Areas with slight growth of bacteria or single colonies were excluded from the clear zone.

2.6. Antibacterial test

The bacterial cells (S. aureus ATCC 6538) were diluted to a concentration of 2.5 × 10⁶ CFU mL⁻¹ after incubating the bacteria in nutrient broth for 37 °C. The diluted bacterial solution was injected into a 0.4 g test sample in an incubator for 2 h. The control sample was set to standard cotton, which proved to have no antibacterial activity. After the desired time point, alive bacteria in the samples were retrieved through vigorous mixing with 20 mL of 0.05 % Tween 80 solution, and then the agar culture medium (cooling down to below 50 °C) was solidified by pouring plate method. The solidified agar was incubated for 24 – 48 h at 37 °C. Afterward, the bacteria concentration was counted using a colony counter by following the equation below.

\[
\text{CFU reduction rate} \times 100 = \left( \frac{C_i - S_i}{C_i} \right) \times 100
\]

(Ci – The number of retrieved bacteria from the control group after desired time point, Si = The number of retrieved bacteria from the experimental group after desired time point). This test was repeated three times for each sample.

2.7. Particle observation set-up using a laser beam

The droplets were observed using a particle observation set-up composed of a particle generator, filter holder box with filter insertion part on top and fan on the bottom, and a 3.5 W laser equipped with beam expanding optics in a single axis to generate a laser screen. The filter holder box is placed inside the black chamber with a slit to penetrate the laser beam. The aerosol particle was generated using saturated NaCl solution, and the created aerosol stream flowed from the bottom to the top. The flow rate was regulated by the RPM controller. For the measurement, the test filter was put on the holder while the intensity of the laser was set to 2.5 W. After the aerosol filled the black chamber (waiting for 30 s), the ejected droplet throughout the filter holder was observed.

2.8. Evaluation of filtration efficiency and pressure drop

The polydisperse aerosol was created from the aerosol generator (Model 3079A, TSI, USA) and continuously flowed into the cylinder acrylic chamber during the filtration test. The component of aerosol particles was oil-based dioctyl sebacate (Sigma-Aldrich). The aerosol filtration efficiency was calculated by comparing the concentration of the downstream and upstream of the filter. The optical particle size spectrometers (Model 3330, TSI, United States of America) were connected on both sides of the cylindrical chamber to measure the distribution of aerosol particle size. The acrylic chamber was separated by a membrane as a filter inserted in the middle of it, and the effective area of the membrane was 0.038 m². The optical particle sizers display the size of aerosol particles from 0.3 μm to 10 μm at the same time. Six tests were performed on each sample, and the mean value of the measurements was used. During filtration measurement, each test was taken for 10 s. The inflow of pre-filtered ambient air into the chamber was generated from the HEPA capsule filter (Model 16022051, TSI, USA) and vacuum pump. The constant airflow was measured and maintained by the airflow meter.
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3. Results and discussion

3.1. Synthesis and chemical structural characterizations

Violacein-embedded electrospun nanofiber filters (VIO-NF) were fabricated by electrospinning of a polymeric mixture containing VIO/PAN dissolved in N,N-dimethylformamide (DMF) with 2:1:9 in weight percent ratio under a constant DC voltage of 12 kV for 3 h with a thickness of 40 μm. As a polar solvent, DMF allows homogeneous solution of VIO, enabling direct mixing with the electrospinning polymer solution. Also, the hydrophobic PAN has repeating units with large dipole moments (~3.6 D) [35,36], providing additional advantages such as good compatibility with VIO, strong moisture resistance, and efficient PM capturing capability. However, note that increasing the concentration of VIO in excess makes the electrospinning process unstable and disrupts the continuous outflow of the electrospinning solution through the needles, leading to the formation of beads and rough morphologies. Therefore, we experimentally determined the optimum content of VIO for VIO-NF to be PAN: VIO: DMF = 1:2:9 (weight ratio). As a control, we additionally fabricated PAN-based nanofiber membranes (PAN-NF) without incorporating VIO. To comparatively study the advantages of the porous nanofiber structure, we prepared VIO film (VIO-F) and PAN film (PAN-F) using by a spin-coating method as reference samples (details in Experimental Section).

Fig. 1B and C show the SEM images of electrospun PAN-NF and VIO-NF with a corresponding photographic image. While the typical PAN-NF is white, the VIO pigment in VIO-NFs gives the membrane a distinct violet color. Compared to PAN-NF (i.e., 650 nm), the fiber diameter in VIO-NF is thicker at around 800 nm with a more rough surface profile, both attributed to the presence of VIO pigments throughout and on the surface of the nanofibers. While the roughness might be attributed to inhomogeneous evaporation of DMF near the VIO-anchored region, there is no notable aggregation of VIO, indicating that the incorporation of VIO does not damage the fiber structures, which can also be demonstrated by the similar mechanical property between VIO-NF and PAN-NF (Fig. S1). Moreover, the energy-dispersive X-ray spectroscopy (EDS) mapping of VIO-NF shows a good distribution of oxygen (O), which also supports the uniform embedment of VIO onto the matrix since oxygen should only be found in VIO while being absent from the chemical composition of PAN (Fig. 1D).

FT-IR analyses were conducted for PAN-NF, VIO-NF, and VIO powder to further confirm that VIO was anchored on a nanofiber structure without chemical degradation during the electrosprinning process (Fig. 1E). We found that VIO-NF has an identical fingerprint with VIO, which exhibits characteristic broadband peaks at 3000–3500 cm⁻¹ coupled with a noticeable band at 3250 cm⁻¹, which corresponds to the O–H stretching band overlapped with the N–H stretching band of a secondary amide, respectively [28]. This result indicates that VIO-NF preserves the chemical structure of VIO, ensuring that VIO-NF would show comparable performance to that of pristine VIO molecules for sterilization against various pathogens. Besides, VIO-NF preserved the characteristic band of C=O at 1710 cm⁻¹, which stems from PAN [37]. Altogether, VIO was successfully immobilized on the surface of the PAN matrix with sterilization functionality.

3.2. Antibacterial activities of VIO-embedded nanofiber filters

Considering that bacteria can survive on textiles for an extended time and cause secondary transmission [5], it is important for PPE materials to not only prevent the passage of bacteria and bacteria carriers but also proactively exhibit biocidal functionalities to significantly decrease the likelihood of potential post-contact transmission of bacteria-based diseases [38]. We evaluated the antibacterial activity of VIO-NF by using a disc-diffusion antibacterial susceptibility test against typical Gram-positive bacteria of Staphylococcus aureus (S. aureus) with pure PAN-NF as a control sample. The VIO-NF and PAN-NF were prepared into discs with a diameter of 11.8 mm and placed on a petri dish with a bed of S. aureus, which was then incubated at 37 °C for 24 h (details in Experimental Section). Afterward, we searched for the inhibition zone in the petri dish, which is the area where bacteria have not grown and/or killed. The VIO-NF showed a clear inhibition zone around the membrane disc with a diameter of approximately 14 mm. On the other hand, PAN-NF virtually showed no inhibition zone, demonstrating the biocidal effects of VIO-NF as a result of the inclusion of VIO (Fig. 2A and 2B). For deeper insights into the antibacterial activity of VIO-NF, the rate of bacterial proliferation was assessed quantitatively by agar plate counting methods (Fig. 2C and Table S1). The PAN-NF allows bacterial growth after 2 h of incubation, which translates to just a 9.6 % reduction rate compared to that of the control (commercial cotton fabric). On the other hand, VIO-NF exhibited effective biocidal characteristics with a reduced rate of 75.5 % within just 2 h, indicating that VIO-NF is quite effective even when benchmarked against previously reported composite polymer nanofibers (Table S2) [18–21]. In particular, the porous nanofiber network of VIO-NF enlarges the contact area for the target bacteria to induce a biostatic effect, immobilizing the bacteria and accelerating cell death. Though a minor concern, C. violaceum (the bacterial strains) could act as an opportunistic pathogen in some cases [39]. As such, nonpathogenic bacterial strains that can produce violacein should be further explored in the future.

3.3. Antiviral activities of VIO-embedded nanofiber filters

In addition to the antibacterial property, we further investigated the antiviral property of VIO-NF by TCID₅₀ experiment (Fig. 3A and 3C and Table S3, details in Experimental Section). The VIO-NF, VIO-F, and PAN-NF (control) were prepared as 19 (mm) disks and placed in culture wells. We applied a 100 μl aliquot of the influenza virus (Influenza A CA/07/2009 pdmH1N1; initial concentration: 1.65 × 10⁵ TCID₅₀ mL⁻¹) and human coronavirus (Human coronavirus: 229E; initial concentration: 7.38 × 10⁶ TCID₅₀ mL⁻¹) into the wells, and the time-dependent inactivation behaviors were assessed by incubating the filters for 1, 2, 4 h. After the set time had elapsed, a virus transfer solution was added to the corresponding samples to extract the remaining live viruses from the matrices. For both influenza and human coronavirus, the VIO-NF
exhibited a remarkably inactivation rate of 98.859 % for influenza and 99.893 % for human coronavirus even within 1 h (Fig. 3B and 3D). These values are noticeably higher than those of PAN-NF (98.091 % for influenza and 92.231 % for human coronavirus), highlighting the particular antiviral functionality of VIO-NF attributed to the VIO that was tightly immobilized on the fiber surface. More surprisingly, after 4 h, VIO-NF has reached an inactivation rate of 99.532 % and 99.999 % for influenza and human coronavirus, respectively, confirming its viricidal capability toward two important strains of respiratory viruses. We found that the VIO content is mainly responsible for viral inactivation since VIO-NF-L (2-fold lower VIO concentration than VIO-NF) exhibited a limited inactivation rate against both viruses (98.455 % for influenza and 99.999 % for human coronavirus after 4 h) compared to VIO-NF (Fig. S2). This observation validates the importance of having the VIO be efficiently exposed to the surface and that it is necessary to fine-tune the concentration of antiviral agents for designing antiviral filters. Besides, VIO-NF has a much higher inactivation rate than VIO-Films (VIO-F, 96.141 % for influenza and 96.931 % for human coronavirus after 4 h). This indicates that the nanofiber matrix has a well-developed open porosity and a large specific surface area, which provides an abundance of anchoring sites where VIO could be exposed and immobilize viruses. Unlike VIO-NFs, most of the VIO in VIO-F was located inside the polymer matrix, making it difficult to fully utilize the VIO for inactivating the viruses (Fig. S3). Therefore, the large aspect ratio and contact area of the nanofibrous scaffold, together with the inherent viricidal properties of VIO, provide a highly effective viricidal performance.

3.4. UV shielding properties

Because the violet-colored VIO pigment is known to have a strong absorbance in the UV range due to the π-conjugated resonance structure of VIO [27], it was hypothesized that VIO-NF filters could selectively absorb UV radiation to offer protection against skin diseases, which is an important consideration, especially for respiratory masks which are
directly exposed to the sun. In general, UV rays are classified into three ranges UVA (315–400 nm), UVB (280–315 nm), and UVC (200–280 nm), in the order of energy intensity [40]. In particular, UVA and UVB are powerful enough to even penetrate the epidermis and, therefore, should be tightly blocked [41]. In this regard, we assessed the response of VIO-NF to UV light in the wavelength range of 280–400 nm. Remarkably, VIO-NF showed substantially reduced transmittance for the entire range compared to pure PAN-NF and typical microfibrous melt-blown filter (MB-MF), where VIO-NF, PAN-NF, and MB-MF rendered UVA protection rates of 93.5, 74.7 and 47.9 %, respectively, and UVB protection rate of 95.4, 94.0, and 52.8 %, respectively (Fig. 4A and Table S4). Note that the better UV shielding effect of PAN-NF compared to that of MB-MF indicates that an electrospun nanofiber structure with well-controlled porosity is preferable for UV absorption since nanofibers are able to refract and scatter incident light in all directions. Furthermore, to gain more insight into the UV shielding efficacy of VIO-NF in the range beyond that of UVA and UVB, we carried out a fluorescence decay test by using rhodamine 6G, a fluorescent dye that responds to deep UV light as deep as UVC (details in Experimental Section). The dye solution was placed at a certain distance from the UV light source (primary peaks at 254 nm and 365 nm corresponding to UVC and UVA, respectively), and then the changes in the fluorescence of the solution after attenuating the UV lamp with VIO-NF, PAN-NF, and MB-MF was recorded (Fig. 4B). Surprisingly, in sharp contrast to PAN-NF and MB-MF, VIO-NF caused a substantial decay in the fluorescence from the rhodamine 6G solution under both UV lights, clearly confirming the outstanding UVC and UVA shielding effect of VIO-NF. It should be noted that the continuous exposure of VIO to UV light can cause its slow decomposition due to the excess energy trapped in the molecules [33,42]. Taken together, we demonstrated that VIO-NF also offers effective UV protection as additional functionality, which makes it suitable for outdoor use and potentially for other UV-sensitive applications.

3.5. Filtration efficiencies and their practical suitability

With the inclusion of VIO having successfully endowed the membrane with promising antiviral, antibacterial, and UV shielding properties, the practical applicability of the VIO-NF as a functional filter for respiratory masks was evaluated by considering its filtration efficiencies and air permeability. Accordingly, we fabricated the VIO-NF membrane with a thickness of 20 μm (VIO-NF20 μm) by setting the electrospinning time to 1.5 h to achieve high filtration efficiency with high air permeability. The filtration performance of VIO-NF against ultrafine particulate matter (PMs) was measured along a pressure gradient by using an air-filtering system consisting of a cylindrical chamber connected to optical particle size spectrometers and a particle generator (Fig. 5A, details in Experimental Section). The polydisperse fine particles created from the particle generator have a size distribution from 0.3 μm to 10 μm, which corresponds to PM 0.3 to PM 10, respectively. Upon measuring the changes in PM concentration with and without the filter, the PM removal rate was calculated according to the following equation (1):

\[
\text{PM removal rate} = \left( \frac{C_0 - C}{C_0} \right)
\]

where \(C\) and \(C_0\) are the concentration of PMs with and without the filter, respectively. Fig. 5B compares the concentrations with and without the VIO-NF as a function of the particle size. Notably, the number of particles upon filtration with VIO-NF was significantly decreased compared to the case without a filter. Consequently, the PM removal rate of VIO-NF was calculated to be 87.8 %, 99.7 %, and 100 % for PM 0.3, PM 1.0, and PM 10 (Fig. 5C), with significant decreases in the particle count for MPSS (most penetrating particle size) with the size around 0.3 μm. The outstanding PM efficiency of VIO-NF results from the increase in the frequency of the interception and inertial impact of PMs against the randomly entangled nanofiber network. Moreover, the excellent filtration efficiency of VIO-NF towards PM 0.3 showed only a slight degradation (ca., 1.3 %) even after 25 days of continuous usage (Fig. S5). This confirms the physical sieving ability of VIO-NF towards ultrafine airborne particles, which is a highly distinctive feature of typical microporous fibers (MB-MF) following the electrostatic capturing mechanism. Also, it was found that the filtration efficiency and quality factor of VIO-NF are very similar to those of PAN-NF (Fig. S6 and S7), proving that the incorporation of VIO does not impair the surface properties which bare PAN nanofibers have. Note that while the thicker 40 μm VIO-NF membrane, obtained by setting the increased electrospinning time to 3 h, can give rise to better PM filtration efficiencies, its pressure drop becomes much steeper in return as depicted in Fig. 5B, leading to deterioration in the breathability and air permeability of the membrane. Therefore, rational control of the processing time is necessary to yield the appropriate fiber density and porosity for preparing the VIO-NF membrane.

As a proof of concept for utilizing VIO-NF filters for PPE applications, a prototype respiratory mask was produced by sequentially assembling three fiber layers, in which the as-prepared VIO-NF layer was inserted between two waterproof microporous polypropylene membranes on either side as an additional barrier against external liquids and/or moisture (Fig. 5D). This three-layered structure covered on either side of the VIO-NF filter can prevent the direct exposure of PAN and VIO to the human body. To visualize the aerosol exclusion efficiency of prototype VIO-NF face masks, we utilized particle observation equipment based on a laser screen, where each of the particles lights up as they scatter the laser beam (Fig. 5E, details in Experimental Section). It is well-known that the transmission of many types of viruses, such as SARS-CoV-2...
occurs through virus-containing aerosol droplets discharged from infected humans via sneezing or coughing, the size of which usually ranges from 0.74 to 2.12 μm [43]. We emulated the aerosol droplets using polydisperse sodium chloride (NaCl) droplets with a diameter of 0.8 to 3.4 μm, produced with a particle generator (Fig. S9). Fig. 5F depicts the particles caught in the laser screen (~3.5 W) after discharge with and without VIO-NF, respectively. In contrast to the case without the filter (Fig. 5F (left)), which displays several particles, the case involving VIO-NF (Fig. 5F (right)) shows almost no aerosol particles, confirming the performance of prototype VIO-NF masks for preventing virus transmission by precluding aerosol droplets. Altogether, the VIO-NF not only passively defends against viral transmission by effectively blocking aerosol particles but also actively kills and/or inactivates any viruses and bacteria that adsorb on the fibrous membrane, offering more powerful protection as PPE compared to currently available solutions for healthcare applications.

4. Conclusions

We demonstrated a nanofiber filter with antibacterial and antiviral violacein compounds immobilized throughout the surfaces of each nanofiber. The violacein component functions to inactivate viruses (e.g., human coronavirus, influenza virus) and bacteria (e.g., S. aureus), while the porous nanofiber membrane physically prevents aerosol and droplet transmission, together with providing the composite filter with enhanced protection against the spread of pathogens. The porous
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by grant No. MCM-2022-N1220047 from KAIST Mobile Clinic Module Project and further supported by the National Research Foundation of Korea Mobile (NRF) grant funded by the Korea government (MSIT) (No. 2021R1C1C2006535 and No. 2020R1A6A1A31077165).

Author contributions

J.L. and J.B. contributed equally to this work, J.L. and J.B. conceptualized and conducted all of the measurements, analyzed the data, and wrote the manuscript. D.Y.Y. evaluated the UV protection test and data analysis. H.B. prepared the violacein and characterization materials property. P.K.B. conducted TCDi30 experiments and edited the manuscript. I.-D. K. supervised the project, procured funds, and revised and edited the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cej.2022.136460.

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