Antiviral Electrospun Polyamide Three-Layered Mask Filter Containing Metal Oxide Nanoparticles and Black Seed Oil

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ABSTRACT: Upon the tremendous spread of coronavirus, there is a need to develop biodegradable, multifunctional, antiviral masks that can be safely used without polluting the environment as conventional surgical masks do. In this study, a three-layered mask filter is designed and fabricated. The first two layers contain electrospun polyamide with dispersed nanoparticles (NPs) of TiO$_2$ and ZnO prepared via breakdown anodization. The third layer is composed of *Nigella sativa* oil (black seed oil) electrospun with polyamide and blended with chitosan to form an effective antiviral three-layered mask filter. The morphological characterization revealed the nanoscale features of the fabricated nanofibers with the ZnO and TiO$_2$ NPs being embedded in the polymeric matrix. The specimens showed good wettability, which is necessary for virus attachment and its subsequent decay. The assembled mask has shown very good mechanical properties. The cytotoxicity results revealed that the proposed mask filter has less cytotoxic effect on the A549 cell line than the commercial KN95 mask filter with maintaining a cell viability of 65.3%. The antiviral activity test showed a variable virucidal effect against human adenovirus on A549 cells. The proposed mask showed the highest effect on the virus followed by PA-ZnO and PA-TiO$_2$ films, which supports the assumption that the used NPs may have broad and promising effects on viruses when combined with the electrospun films.

INTRODUCTION

To inhibit the rapid transmission of new variants of Covid-19, various recommendations have been proposed, including the continued instruction of wearing masks. The commercially used masks are cloth masks, surgical masks, and respirator masks (P2, KN95, and N95). Cloth masks have very low protection and surgical masks have very low efficacy against bacteria and viruses. On the other hand, respirator masks, which offer higher protection, are very expensive to use on a daily basis.† Also, the utilization of daily disposable, non-degradable face masks has resulted in an increase of environmental wastes.‡ This necessitates the design and fabrication of biodegradable mask filters that decrease these plastic disposables.§ The rapid transfer of Covid-19 caused a great demand to produce multifunctional mask filters to maintain the balance between two functional pathogenic activations by improving materials’ virucidal properties, while maintaining the air filtration efficiency to the pathogens. Therefore, fabricating a multilayered mask filter with different active materials should achieve those desired characteristics. In this regard, nanofibrous materials exhibit effective filtration properties due to their high surface area-to-volume ratio with a controllable pore size. The main technique to produce nanofibrous mats is the electrospinning technique, which is an effective multipurpose technique to develop micro and nanofiber membranes with a porous structure and controlled fiber dimensions, pores, and morphology for different applications.¶ In comparison to conventional filters, electrospun air filters exhibit reduced pore sizes (sub-micrometers to several micrometers) that can capture small airborne particles.§

Polyamide (Nylon) is a cheap polymer, which consists of amide groups separated by methylene sequences that can be electrospun with good mechanical, thermal, and chemical properties.¶ To enhance the biodegradability of polyamide, it was blended with chitosan that has antibacterial and antiviral properties besides its biodegradability, biocompatibility, and nontoxicity.¶,¶,¶,¶ Moreover, nanoparticles (NPs) were shown to enhance the properties of polymeric materials due to their high surface area-to-volume ratio. Specifically, inorganic nanomaterials have shown significant antibacterial and antiviral activities at very low concentrations.¶,¶ Some of them are nontoxic and may contain mineral elements that are vital for the human body.¶ Of those nanomaterials, titanium dioxide is an effective antibacterial and antiviral that exhibits biocompat-

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ibility and safety with relatively low cost. However, titanium dioxide NPs with sizes ranging from 2 to 5 nm were shown to cause health risks due to their ease of inhalation. Therefore, it is better to use bigger titanium dioxide NPs, especially in the nanotube (NT) form with one of their dimensions in the micro range, to decrease the health risks. Titanium dioxide NTs can be easily synthesized in the powder form via rapid breakdown anodization. Titanium dioxide offers a number of advantages, including low human toxicity and good ultraviolet (UV)-activated viral suppression, thus it has been chosen as a suitable model to study the antiviral activity of a plethora of viruses. Various studies demonstrated the ability of TiO$_2$ against respiratory viruses such as influenza virus, respiratory syncytial virus, or other types such as human norovirus, herpes simplex virus 1, and hepatitis B antigen (HBsAg).

On the other hand, zinc is an essential element of many enzymes in the human body and can induce cell proliferation. Also, zinc oxide NPs are inorganic metal oxides that are safe and can be used safely in medicine, packaging, and sunscreens, and as antimicrobial agents. The biological effects of ZnO also depend on its particle size and morphology. ZnO NPs have a wide effect on many bacterial strains by destroying the bacterial cell membrane and accumulating in the cytoplasm. ZnO has also been proven to be an antiviral material for many viruses. ZnO has virostatic potential against some viruses such as herpes simplex virus (HSV), where ZnO inhibits the electrostatic interaction between the virus and the host cell. Moreover, ZnO was shown to block HSV-2 entry into the target cells and provide a significant therapeutic efficacy by lowering the quantity of plaques that are formed as a result of the treatment. In another study on influenza virus, ZnO-polyethylene glycol (PEG)-NPs were shown to have a stronger antiviral effect along with lower cytotoxicity than ZnO NPs, confirming that the surface PEGylation of NPs plays a key role in the enhancement of the antiviral activity of ZnO against H1N1 influenza virus.

*Nigella sativa*, also known as black cumin seed, belongs to the Ranunculacea family. It contains various active compounds like trepans such as thymoquinone (TQ), dithymoquinone, carvone, limonene, trans-anethol, and p-cymene, indazole alkalds like nigellidine, nigellicine, and isoquinoline alkaoids including nigellicmine, nigellicine-N-oxide, and $\alpha$-hederin. It is well known in old medicine remedies and used to treat asthma, common cold, headache, and nasal congestion. It possesses antibacterial, antioxidant, anticoagulant, immunomodulatory, bronchodilator, anti-inflammatory, antihistaminic, antiviral, antitussive, antipyretic, and analgesic activities. Many studies reported that it could inhibit Covid-19 virus.

Herein, we demonstrate the design and fabrication of a degradable three-layered electrospun bifunctional air mask filter that has active antiviral activity besides its high filtration efficiency. The outer active layer of the filter is composed of electrospun polyamide with incorporated NTs of TiO$_2$. The middle layer is composed of ZnO NPs embedded in the polyamide fibers. The last layer, which will be in contact with the face and the mouth surface, must also possess antiviral properties be smooth, and be the safest layer. Therefore, this last layer was composed of an electrospun layer of polyamide with chitosan containing black seed oil. The mask components are cost-effective and easy to be reproduced in large scale production.

### EXPERIMENTAL SECTION

#### Materials.

All chemicals and reagents were used without any further purification, except zinc and titanium foils. Pure metallic zinc and titanium foils (purity: 99.9%, 1 cm × 2 cm) were purchased from Alfa Aesar and platinum foil was purchased from Sigma-Aldrich. (70%). Perchloric acid was purchased from Central Drug House (P) Ltd., India. Formamide and NH$_4$F were purchased from Sigma-Aldrich. Polyamide was purchased from Roth and black seed oil was purchased from the Oil Extraction Unit-National Research Centre, Cairo, Egypt.

**Fabrication of TiO$_2$ NTs.** TiO$_2$ NTs were prepared via the breakdown anodization of Ti foil. Ti foil was sonicated in ethanol, acetone, and deionized (DI) water for 30 min to remove any surface grease. In the anodization process, platinum and Ti foils (0.1 mm thick) were used as counter and working electrodes, respectively. The electrodes were immersed in 0.1 M perchloric acid (HCO$_3$) electrolyte solution in an ice bath at 0 °C at 20 V with a DC power supply. The distance between the counter and working electrodes was 1.0 cm. During this process, Ti pieces were completely transformed to a fine powder of TiO$_2$ NTs with time. The powders were washed thoroughly with DI water and ethanol many times to ensure that there was no residual acid remaining, and then left to dry in desiccators for 4–5 days. The wet powder of TiO$_2$ NTs was thermally annealed at 450 °C with a heating and cooling rate of 10 °C/ min for 4 h.

**Fabrication of ZnO NPs.** The rapid breakdown anodization was performed using zinc foil as the working electrode and platinum foil as the counter electrode. The samples were cleaned by ultrasonicating them in acetone, ethanol, and distilled water for 60 min. The experiment was conducted in an ice bath at 0 °C in formamide-based electrolytes containing 0.2 M NH$_4$F, 1.5 vol % HCO$_3$, and 3 vol % H$_2$O at an applied voltage of 25 V for 1 h. The distance between the counter and working electrode was 1.0 cm. During this process, Zn pieces were completely transformed to white powder of ZnO NPs. After the breakdown anodization, the powders were washed thoroughly with DI water and ethanol many times to ensure that there was no remaining acid, and then left to dry in desiccators for 4–5 days. The wet ZnO NPs powder was thermally annealed at 450 °C with a heating and cooling rate of 10 °C/ min for 4 h to enhance the stability and electrical conductivity.

**Fabrication of the Filters by the Electrospinning Technique.** All the prepared solutions were then injected into 1 mL syringes with 21 G needle under 20 kV applied voltage and at 12 cm tip to collector. The voltage was adjusted using a Glassman High Voltage Series (voltage range 0–20 kV). Syringe Pump Series 100 regulated the flow rate of the solution. Electrospinning was done at room temperature (25 °C) and at a relative humidity of 60 ± 2%. The different compositions used in this study are listed in Table 1.

**Optimum Oil Ratio Study.** Two concentrations of black seed oil (15 and 30%) were added to 20% polyamide polymeric solution dissolved in 70:30 (FA/AA) solvent systems.

**Characterization.** The prepared NPs were characterized by field-emission scanning electron microscopy (FESEM) with energy dispersive X-ray (EDX) spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and X-ray powder
diffraction (XRD) to assure the preparation of the NTs. The morphology, pore size, and fiber size of the prepared electrospun films were studied by FESEM (Zeiss). The change in the functional groups was studied by FTIR (PerkinElmer Spectrum 10.5.4). Surface hydrophilicity was estimated by the sample was performed using an SDTQ600 analyzer with a scanning calorimetry/thermogravimetric analysis (TGA)) of microscopes, manufactured by SDL-UK. The contact angle in the functional groups was studied by FTIR (PerkinElmer Universal Testing Machine (Lloyd Instruments Ltd. LR10 K, Hampshire, UK) at a stroke rate of 10 mm per minute. The dimensions of the samples were 10 mm width and 80 mm length with a gap length of 2 cm. Each mat was tested five times and the results were averaged.

**Degradation and Water Uptake.** The films were cut into 2 × 1 cm² samples and weighed (Wᵢ). Afterward, the films were soaked in 15 mL of phosphate buffer solution (PBS) and incubated at 37 °C for 1, 3, 7, 14, and 30 days. After each prescribed day, the mats were taken out and dried by a filter paper to absorb all the surface solutions, then the samples were weighed (Wᵢ). The samples were weighed again after drying for 24 h at room temperature (Wᵢ). The percentage weight loss and the water uptake percentage were calculated according to the following relations:

\[ \text{weight loss (\%)} = \frac{Wᵢ - Wᵢ}{Wᵢ} \times 100 \]  
\[ \text{water uptake (\%)} = \frac{Wᵢ - Wᵢ}{Wᵢ} \times 100 \]

**Virus Stock Preparation and Plaque Assay.** The human adenovirus type 2 (HAdV-2) strains were kindly provided by Prof. Dr. Celia Barardi, Federal University of Santa Catarina, Brazil. The human lung carcinoma cell line (A549 cells), obtained from SPL Life Sciences (Gyeonggi-do, Korea) and France, were used to grow the cells. Six-well plates were incubated at 37 °C for 48 h for each replicate.

**Antiviral Testing.** The virucidal activity of the tested films was evaluated by the method reported by Haldar et al. with slight modifications. Films of 1 cm × 1 cm were individually placed at the bottom of the wells of a 6-well plate. To each sample, a 2 mL aliquot of the virus suspension (10⁶ PFU/mL) was added and incubated at 23 ± 1 °C in an incubator shaker for 24 h. A nontreated virus suspension was added to one well and used as the positive control. After incubation, 0.5 mL of the treated films were withdrawn separately and diluted with 0.5 mL of DMEM. In parallel, the A549 cell line was seeded in a 48-well plate at a concentration of 2.5 × 10⁴ cells/well and at 37 °C under 5% CO₂. The virus was harvested after 2 days of incubation by three freeze–thaw cycles and centrifugation at 3000 g for 20 min at 4 °C. The supernatant was collected and the viral titer was determined by plaque assay. The HAdV-2 plaque assay was performed on A549 cells seeded into 6-well plates and incubated for 24 h at 37 °C under a 5% CO₂ atmosphere according to the method reported by Cromeans et al. The cell monolayers were then infected with 400 μL of appropriate serial dilutions of the virus suspension and the plates were incubated for 1 h at 37 °C, with gentle shaking every 15 min, to allow for the virus attachment. After the removal of the inoculate, the cells were overlaid with 2 mL of Eagle’s minimum essential medium (MEM) containing 1% agarose, 2% FBS, 1% sodium bicarbonate, 0.1 mg of kanamycin/mL, 0.05 mg of gentamicin/mL, 15 mM HEPES (pH 7.7), and 2 mM l-glutamine (Invitrogen). After 48 h of incubation at 37 °C and 5% CO₂, the plates were fixed and stained with 10% formaldehyde and crystal violet. The plaques were then counted and the virus titer was calculated using the following equation:

\[ \text{PFU/mL} = \frac{\text{Dilution factor} \times \text{Volume (mL) of virus inoculum added}}{\# \text{Plaques} \times \text{Volume (mL) of virus inoculum added}} \]  
(3)
incubated for 24 h at 37 °C under 5% CO₂. The culture medium was removed from the wells, and the antiviral assays were carried out by adding 100 μL to each withdrawn mixture onto the cells and incubated at 37 °C under 5% CO₂ for 2 h. Cells infected with the nontreated virus were used as the positive control and noninfected cells without any treatment were used as the negative control. The mixed solution was discarded from the wells, and the cell culture was rinsed by the medium and incubated with 500 μL of test medium at 37 °C under a 5% CO₂ atmosphere for 24 h. The supernatants were removed, and cell layers were washed with PBS (Biowest, France) and incubated with 100 μL of MTT (3-(4, 5-dimethylthiazolyl)-2, 5-diphenyl-tetrazolium bromide, Sigma) (5 mg/mL) for another 4 h at 37 °C according to the manufacturer’s protocol. The supernatants were discarded carefully and 500 μL of DMSO (Sigma-Aldrich) were added

Figure 1. (a, b) FESEM images and (c, d) XRD patterns of the synthesized TiO₂ NTs and ZnO NPs.

Figure 2. FESEM images of the electrospun polyamide containing (a) 0%, (b) 15%, (c) 30% oil, (d) bare polymer, (e) polymer + 5% Cs, (f) polymer + 15% oil, (g) polymer + 15% oil + 5% Cs, (h) polymer + ZnO, and (i) polymer + TiO₂.
for each well and incubated for 30 min at 37 °C to dissolve the formed formazan crystals. After incubation, 100 μL of the solutions were transferred to a clean 96-well plate, and the OD of the resulted solutions was quantified at 570 nm by an enzyme-linked immunosorbent assay reader. The infectivity of HAdV-2 after treatment with the tested films was described as cell viability percentage using the following formula [(A − B)/C × 100], where A, B, and C imply to the mean three absorbance values of each treated sample, negative control for Viable cells, respectively. All the experiments were assayed in triplicates and three OD reads were measured for each replicate.

**Statistical Analysis.** The values reported are the average ± standard deviation. Statistical analyses were performed with the one-way analysis of variance (ANOVA) test, attached by excel software. Values of p < 0.05 were considered statistically significant.

## RESULTS AND DISCUSSION

**Fabrication and Characterization of the Layers.** The morphology of the anodized Ti foil is shown in Figure 1a, revealing the formation of agglomerated tiny TiO$_2$ NTs with an average diameter of 20 ± 6 and length of 788 ± 382 nm. Similarly, ZnO, Figure 1b, appears like agglomerated NPs with a size of 76 ± 26 nm. The EDX spectra confirm the existence of Ti, Zn, and O elements, Figure S1. The XRD spectra of the prepared TiO$_2$ NTs, shown in Figure 1c, indicate the formation of anatase TiO$_2$. The small peak at 2θ = 44.5° is related to the (200) plane. The peak at 2θ = 25° corresponds to the (101) plane and the small peak at 38° corresponds to the (004) plane. The peaks at 2θ = 53, 55, 63, and 70° correspond to the (200), (105), (204), and (220) planes, respectively, which are consistent with the TiO$_2$ JCPDS card no. 21-1272. On the other hand, the XRD patterns of the as-prepared ZnO NPs (Figure 1d) are consistent with the values reported in the database of ZnO (JCPDS card no. 792205). The sharp peak at 2θ = 36° corresponds to the (100) plane. The peak at 33° corresponds to the (002) plane and the peaks at 2θ = 35, 48, 58, and 62° correspond to the (101), (102), (110), and (103) plans, respectively.

The FESEM images of the electrospun polyamide with 0, 15, and 30% oil are shown in Figure 2a–c. Note that the addition of oil causes an increase in the fiber diameter. At 30% oil, the fibers exceeded the maximum loading capacity due to the increased viscosity, resulting in their agglomeration on the surface with many beads. Therefore, we decided to choose the 15% oil-polyamide for the fabrication of the filter. The FESEM images of the prepared electrospun polyamide with the different components are shown in Figure 2d–i. All the electrospun fibers are shown to be homogeneous and smooth nanoscaled fibers. All the nanofibrous structures showed a large surface area-to-volume ratio, which makes the mats more reactive. The fiber diameter ranges from 25 to 200 nm with pore size distribution from 100 to 400 nm. Upon the addition of 5% chitosan (Cs), the fiber diameter appears to be finer with lower pore sizes (Figure 2e), in agreement with those reported by Fazeli et al. Upon the addition of the oil, it became larger with bigger pore sizes (Figure 2f). The addition of both chitosan and oil (Figure 2g) resulted in a similar fiber with a similar pore size distribution to the initial electrospun polymeric mat, which constitutes the first filter layer. The addition of ZnO caused a slight increase in the mean pore size diameter from 83 ± 31 nm in the case of pristine electrospun polyamide to 111 ± 50 nm and an increase in the fiber diameter from 56 ± 27 to 71 ± 27 nm (Figure 2h). The third layer, TiO$_2$ (Figure 2i), showed also a slight increase in pore size with a mean pore size of 159 ± 82 nm and mean fiber diameter of 57 ± 22. The change in fiber diameter and pore size diameter may be attributed to the change in viscosity and the interaction between the added NPs and the polymeric chains.

The FTIR spectra of the prepared materials are shown in Figure 3. The spectra of TiO$_2$ (Figure 3a) showed bands at 3333 and 1634 cm$^{-1}$, which are attributed to the surface adsorbed water and hydroxyl groups, respectively. The bands at 450 and 726 cm$^{-1}$ are attributed to the O−Ti−O bonding. The FTIR spectrum of the annealed ZnO NPs is also shown in Figure 3a. The sharp peak at 489 cm$^{-1}$ is attributed to the ZnO stretching bands. The bands from 600 to 1700 cm$^{-1}$ correspond to C=O, C−O, and C−H vibrations. The FTIR of the prepared NPs and the oil and their electrospun fibers is shown in Figure 3b. The FTIR spectrum of the Nigella sativa oil shows a weak absorption peak at 3009 cm$^{-1}$ that corresponds to the C−H stretching of the vinyl group. Two bands are observed at 2923 and 2854 cm$^{-1}$, which can be...
Table 2. Mechanical Properties of the Prepared Films

| sample name          | Young’s modulus (MPa) | tensile strength (MPa) | elongation at break (%) |
|----------------------|-----------------------|------------------------|-------------------------|
| polymer              | 5.08 ± 0.3            | 6.08 ± 0.38            | 28.99 ± 2.88            |
| polymer + Cs         | 2.84 ± 0.99           | 4.19 ± 0.29            | 17.58 ± 4.61            |
| polymer + Cs + oil   | 4.06 ± 0.32           | 4.29 ± 0.46            | 28.16 ± 4.38            |
| polymer + ZnO        | 4.42 ± 0.15           | 4.62 ± 0.51            | 12.38 ± 1.60            |
| polymer + TiO₂       | 2.98 ± 0.34           | 3.67 ± 0.13            | 50.08 ± 2.75            |
| mask filter          | 3.86 ± 0.72           | 4.97 ± 0.17            | 24.29 ± 3.05            |

Figure 4. (a) Mechanical properties and (b) TGA of the prepared films.

assigned to the C–H stretching of an aliphatic group, indicating the existence of methyl and isopropyl substituents. Moreover, intense bands are observed at 1746 and 1714 cm⁻¹, which correspond to the C=O stretching of the forster and ketone groups, respectively. Also, an absorption band was observed at 1659 cm⁻¹, belonging to the C=O stretching of TQ. The two peaks at 1463 and 1378 cm⁻¹ can be related to C–H. The weak peak at 1165 cm⁻¹ owing to the C–O group and the band at 1099 cm⁻¹ owing to the ==C–H bending group is observed. The strong peak at 672 cm⁻¹ is related to the C–H bending of an aliphatic group.

Table 3. Contact Angle of the Three Layers

| sample name            | contact angle (°) |
|------------------------|-------------------|
| polymer + Cs + oil     | 68.08 ± 9.47      |
| polymer + ZnO          | 60.84 ± 3.06      |
| polymer + TiO₂         | 56.61 ± 6.03      |

and TiO₂ caused a change in both tensile strength and elongation due to the incorporation of the NPs inside the fibers. TGA was performed to elucidate the thermal stability of the fabricated filter layers, Figure 4b. The electrospun polyamide with chitosan and black seed oil show lower stability as they degrade at a lower temperature than ZnO and TiO₂.

Water Uptake and Biodegradation. As SARS-CoV-2 spreads as watery droplets from an infected person that are aerosolized to infect another person or settle on surfaces for a while, the hydrophilicity of the material is a crucial factor that determines functionality of the face mask material. Therefore, the contact angle of the fabricated films has been determined as listed in Table 3. All the three layers show a hydrophilic nature, which ensures the inactivation of the virus by broadening of the water droplets. Note that the addition of ZnO and TiO₂ enhanced the hydrophilicity of polyamide. SARS-CoV-2 is a lipid bi layer-enveloped virus containing surface glycoproteins, and NP interactions with SARS-CoV-2 viral membranes may compromise surface proteins including the spike glycoprotein. Increasing the surface area-to-volume ratio and hydrophilicity should facilitate the interaction of surface NPs with the viral membrane leading to its inactivation.

Analysis of water uptake measurements of the fabricated mats after 24 h indicates the ability of the mask layers to absorb water and humidity. The water uptake percentage of all the samples is shown in Figure 5a. Note that the highest water uptake values were observed for polymeric mats loaded with ZnO and TiO₂ due to their high porous structure as observed from the FESEM imaging. Those porous structures help to adsorb water into their inner pores aided by the hydrophilic nature of ZnO and TiO₂. The degradation behavior of the different electrospun mats is shown in Figure 5b. Nearly, all the samples have shown very high stability due to the hydrophobic nature of polyamide, which indicates that it will not degrade quickly by time and sterilization.

Cytotoxicity of the Films on A549. The cytotoxicity patterns are variable among the synthesized films and reveal a decrease in the viability of A549 cells, Figure 6, with viability above 60%. As the used cell line is a cancerous cell line, the bare polyamide membrane showed the highest viability of 72%. Upon the addition of either black seed oil or chitosan, the viability slightly decreased due to their anticancerous properties. However, the addition of both black seed oil and chitosan together caused a slight increase in viability. It seems that
interaction between chitosan and oil led to a decrease in the anticancerous properties. Also, the increased hydrophobicity resulted from the semioily surface is not favorable for cell growth. The proposed film (Mask) was less cytotoxic and maintained a cellular viability of 65.3% followed by ZnO (65%) and TiO$_2$ (63%) films. Note that the commercial mask (KN95) decreased the cellular viability to (27.4%). According to the results, each layer of the proposed mask had a different cytotoxic effect on the cells, but when they were combined together, their effect was less cytotoxic, and the cell viability remained higher.

**Antiviral Characteristics of the Films.** To evaluate the virucidal activity of the proposed mask, HAdV-2 was used as a virus model to represent the respiratory viruses as it is responsible for approximately 5% of acute respiratory infections, including bronchitis/bronchiolitis and pneumonia.52–54 NPs have distinctive chemical and physical properties, which allow them to interact with biological targets as viruses, making them promising antiviral materials.55 Different strategies have been reported to fabricate antiviral materials, including the development of compounds that react with the host cell by blocking the virus receptor or those that affect the virus itself by binding or destroying the binding sites on the virus surface.56,57 The antiviral activities of the prepared films were evaluated against HAdV-2 using the indirect evaluation of A549 cells viability by MTT assay. The tested films were mixed equally with fixed viral concentration solutions (10$^6$ PFU/mL) to evaluate the effect of film components in order to reduce the initial viral load used, Figure 7. Chitosan showed indirect viral inhibitory properties due to its propensity to trigger innate immunity in cells.58 However, the results showed that the inhibitory effect of chitosan on the virus was less than the effect of the proposed mask. This reveals that most of the virucidal effect of the mask is due to the addition of ZnO and TiO$_2$ NPs, which maintain cell viability at high levels of 54.8 and 49.1%, respectively. The black seed oil enhanced the antiviral activity of the polyamide as well as the polyamide combined with chitosan due to their virucidal activity.59 Note that the antiviral activity of the proposed mask (all three layers) showed the highest effect on the virus, with the highest cell viability (60.9%) followed by PA-ZnO and PA-TiO$_2$ layers. The prepared mask was made from different layers that consist of NPs, ZnO, and TiO$_2$, as well as black seed oil and chitosan, while the commercial mask was probably made of polymeric materials without the use of the NPs. This explains the high cytotoxicity of the prepared mask compared to the commercial mask as shown in Figure 6. All mask layers together showed some cytotoxicity at a moderate level against A549 cells, where at this level of cytotoxicity the prepared mask was able to inhibit the virus propagation as shown in Figure 7. These findings support the
assumption that the used NPs have broad and promising effects on viruses when combined. On the other hand, the results were compared to the positive control, which consists of the initial virus load (10⁶ PFU/mL) that was incubated under the same conditions and showed the lowest percentage of cell viability (29.9%). The negative control was used as a noninfected cell, which showed high cell viability (94.3%).

To understand the antiviral activity of the proposed mask, the antiviral effectiveness of the mask components was demonstrated along with other studies on DNA and RNA viruses. The chitosan used in the mask layer was reported to have indirect viral inhibitory properties due to its propensity to trigger innate immunity in cells. In agreement with these results, our study showed that the inhibitory effect of chitosan on the virus was less than the effect of the proposed mask, which confirms that the virucidal effect of the mask can be ascribed to TiO₂ and ZnO NPs. The virucidal potential of ZnO against some viruses like HSV has already been reported, which was attributed to its ability to inhibit the electrostatic interaction between the virus and the host cell. Another study showed that ZnO-PEG-NPs have a stronger antiviral effect on influenza virus along with lower cytotoxicity compared to ZnO NPs, confirming that the surface PEGylation of NPs plays a key role in the enhancement of the antiviral activity. On the other hand, various studies demonstrated the ability of TiO₂ against respiratory viruses like influenza virus, respiratory syncytial virus, or other types like human norovirus, HSV-1, and HBsAg. The proposed photocatalysis mechanism affects the viruses and led to different effects including damage to the viral membrane, protein capsid, and RNA. The same destruction mechanism of the surface HBsAg was reported for the photocatalytic effect of TiO₂ NPs under the action of UV and sunlight.

## CONCLUSIONS

One of the most important reasons for the emergence of the Covid-19 pandemic outbreak is the transmission of the virus through the respiratory system, which led to the mandatory obligation to use disposable masks that resulted in significant economic costs and environmental impacts. In the present study, we proposed and demonstrated a disposable mask with promising antiviral activity against the respiratory viruses. The proposed mask materials are composed of electrospun polyamide fibers containing chitosan with black seed oil and NPs of zinc oxide (ZnO) and titanium dioxide (TiO₂). Adeno virus was used as a respiratory virus model. The electrospun layers have a hydrophilic surface, which disperse the droplets from the infected person on the electrospun membrane, in addition to its high surface area-to-volume ratio, leading to virus inactivation by its active components. In addition, the electrospun layers showed good mechanical properties, making them durable for use to construct mask filters. The electrospun layers containing chitosan, black seed oil, and the TiO₂ and ZnO NPs showed higher antiviral activity than the bare polyamide layer. As a result, the proposed filter has bifunctional antiviral activity by air filtration through the tiny pores and also by surface deactivation of the virus.

## ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c06611.

EDX spectra of the prepared TiO₂ NTs and ZnO NPs (PDF)

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B.E. contributed equally to this study.

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