PROTEIN-BASED NANOFABRICS FOR MULTIFUNCTIONAL AIR FILTERING

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of HAMID SOUZANDEH find it satisfactory and recommend that it be accepted.

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PROTEIN-BASED NANOFABRICS FOR MULTIFUNCTIONAL AIR FILTERING

Abstract

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With the fast development of economics and population, air pollution is getting worse and becomes a great concern worldwide. The release of chemicals, particulates and biological materials into air can lead to various diseases or discomfort to humans and other living organisms, alongside other serious impacts on the environment. Therefore, improving indoor air quality using various air filters is in critical need because people stay inside buildings most time of the day. However, current air filters using traditional polymers can only remove particles from the polluted air and disposing the huge amount of used air filters can cause serious secondary environmental pollution. Therefore, development of multi-functional air filter materials with environmental friendliness is significant. For this purpose, we developed “green” protein-based multifunctional air-filtering materials. The outstanding performance of the green materials in removal of multiple species of pollutants, including particulate matter, toxic chemicals, and biological hazards, simultaneously, will greatly facilitate the development of the next-generation air-filtration systems. First and foremost, we developed high-performance protein-based nanofabric air-filter mats. It was found that the protein-nanofabrics possess high-efficiency multifunctional air-filtering properties for both particles and various species of chemical gases. Then, the high-performance natural protein-based nanofabrics were promoted both mechanically and functionally by a textured cellulose paper
towel. It is interestingly discovered that the textured cellulose paper towel not only can act as a flexible mechanical support, but also a type of airflow regulator which can improve the pollutant-nanofilter interactions. Furthermore, the protein-based nanofabrics were crosslinked in order to enhance the environmental-stability of the filters. It was found that the crosslinked protein-nanofabrics can significantly improve the structure stability against different moisture levels and temperatures, while maintain the multifunctional filtration performance. Moreover, it was demonstrated that the crosslinked protein-nanomaterials also possess antibacterial properties against the selected gram-negative and gram-positive bacteria. This provides a cost-effective solution for advanced “green” nanomaterials with excellent performance in both filtration functions and structure stability under varying environment. This work indicates that protein-based air-filters are promising “green” air-filtering materials for next-generation air-filtration systems.
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Dedication

To my Mom and Dad who always supported me and encouraged me to succeed.

To Niloo far AKA Doozi, my beautiful soulmate and love of my life, whom without her I would have finished my PhD two years earlier.

To my best friend Mehrdad who had always been there for me.
CHAPTER ONE: INTRODUCTION

1.1 AIR POLLUTANTS AND AIR FILTERING MATERIALS

1.1.1 Introduction to Air Pollutants

Air pollution has been a growing concern, and the cleaning of polluted air is becoming increasingly challenging mainly because of the complicated composition of the pollutants. The polluted air contains particulate matter (PM) of various sizes, chemical vapors, bacteria/viruses, and so on. The release of toxic chemicals, particulates and biological materials into air has led to various diseases or discomfort to humans and other living organisms. It is known that air pollution is responsible for numerous premature deaths, alongside their irreversible impacts on the environment. According to the 2009 and 2012 World Bank report, more than 60% of Americans live in air quality levels that are potentially detrimental to their health,\(^1\,^2\) and could even cause damages to agricultural crops. In general, due to the complexity of the pollution sources, including fuel combustion, factory plants, tobacco smoke, dust, etc. the composition of pollutants in polluted-air is extremely complicated. Figure 1.1 shows the classification of air pollutants. Particle Matter (PM) is usually categorized into two groups, PM\(_{2.5}\) and PM\(_{10-2.5}\) which denote particles with aerodynamic diameters smaller than 2.5 μm and between 2.5–10 μm, respectively.\(^3\,^4\) PM\(_{2.5}\) particles are the critical particulate pollution to be filtered due to their ability to penetrate into human lungs and bronchi.\(^3\,^\text{–}\,^\text{11}\) Most of the particulate pollutants are made of organic compounds, such as carbon derived species (e.g., CO\(_2\) and CO) and sulfur- and nitrogen-based inorganic compounds (e.g., SO\(_2^{2-}\), SO\(_{4}^{2-}\), NO\(_3^{-}\)).\(^12\,^\text{–}\,^14\) Additionally, there are also various toxic gaseous molecules, such as nitrogen dioxide (NO\(_2\)), methane (CH\(_4\)), carbon monoxide (CO), formaldehyde (HCHO), and volatile organic compounds (VOCs).
Bio-aerosols such as bacteria, viruses, and fungi represent another type of primary pollutant which greatly increases the risks to the public health since they can easily spread over by polluted air very quickly. These biological pollutants can cause both chronic and acute diseases that can be allergenic, infectious, toxigenic, etc. This was proved by the significant discovery of the Severe Acute Respiratory Syndrome (SARS) and Middle Eastern Respiratory Syndrome (MERS). The existing pollutants in the air produced from different sources (such as petrochemical and allied industries) can participate in various photochemical reactions in the
atmosphere and create significant environmental hazards.\textsuperscript{21,22} Air pollution outside makes improving indoor air quality increasingly desired. Therefore, more and more buildings incorporate air filtration protection in their heating, ventilation, and air conditioning systems. Hence, the demand for high-efficiency air-filtering materials that are able to simultaneously capture hazardous particles, chemical gases, and biological contaminants is increasing dramatically.

\subsection*{1.1.2 Classification of Air Filters}

Air filters are the most common used devices to remove pollutants from the air. They are widely used in various areas such as automotive industries, residential, general commercial, hospitals and general surgeries. Traditionally, air filters are made of porous films, such as non-woven fibrous mats with randomly oriented micro size fibers. The fibers are made of chemically synthesized or petroleum-based materials, such as polypropylene and fiberglass. There are three main types of air filters: particulate, chemical and anti-microbial as shown in Figure 1.2. Particulate air filters only target particles with different sizes. High efficiency particulate air filters (HEPA) are one of the most commonly used air filters. Chemical air filters contain high loadings of activated carbon that can absorb small chemical molecules. Antimicrobial air filters usually include high loadings of expensive nanoparticles, such as silver or titanium oxide that provide their antibacterial properties. However, PM and gas filters give negligible protection against each other’s hazard.\textsuperscript{23,24} Therefore, in current air filtering systems, in order to provide a high quality air downstream of the air filter, utilizing all different types of air filters such as regular particulate, HEPA, activated-carbon, antimicrobial, etc. at the same time are critically needed. Using multiple filters could increase the pressure drop downstream of the filter and, as a result, consumption of a huge amount of energy and cost for active air exchange.
1.1.2.1 Particulate Air Filters

Particulate air filters are commonly made of chemically synthesized or petroleum-based raw materials, such as polypropylene and fiberglass and are designed to target particles with different sizes. High efficiency particulate air filters (HEPA) are one of the most commonly used air filters. The Department of Energy (DOE) has set certain requirements for a filter in order to be categorized as a HEPA filter: (1) possessing more than 99.97% removal efficiency of 0.3 µm particles; (2) maintaining a pressure drop lower than 1.3 in.H$_2$O (320 Pa).\textsuperscript{25} It is noted that these conventional materials provide very limited chemical functionality, resulting in insufficient interactions with
pollutants. Moreover, disposing of used air filters made of these materials can cause further
environmental pollution as most of them are not environmentally friendly. Particulate filtration is
regularly defined by three terms: (1) filtration efficiency; (2) air flow resistance (also known as
pressure drop); (3) quality factor (also known as the figure of merit).\textsuperscript{23} The filtration efficiency
and pressure drop can be determined using the experimental setup shown in Figure 1.3. Pressure
drop is defined as the air pressure difference between upstream and downstream of the air filter
which is critical to be minimal to avoid excess energy consumption.\textsuperscript{26-28} The filtration removal
efficiency ($\eta$), air flow resistance ($\Delta P$), and quality factor ($Q$) can be determined via equation (1)-
(3):

$$\eta = \frac{C_p - C_c}{C_p} \quad (1)$$

$$\Delta P = P_{up} - P_{down} \quad (2)$$

$$Q = -\frac{\ln(1-\eta)}{\Delta P} \quad (3)$$

where $C_p$: pollutant concentration in the polluted air sample before testing; $C_c$: pollutant
concentration downstream of the filter after filtration testing; $\Delta P$: air flow resistance (pressure
drop); $P_{up}$: upstream air flow pressure; $P_{down}$: downstream air flow pressure; and $Q$: quality factor.

The particulate filtration can also be categorized in terms of filter resistance: most penetrating
particle size (MPPS) and minimum efficiency reporting value (MERV).\textsuperscript{16} This characterization
type is based on breathability of the filter which directly represented by the pressure drop.\textsuperscript{23,29,30}
MPPS is the particle size at which none of the capturing mechanisms is predominant and able to
filter it. For most filters, MPPS is in between 0.1-0.3 $\mu$m.\textsuperscript{31} MERV is minimum fractional particle
size efficiency in three various particle size ranges. The fraction of aerosol penetration (P) can be calculated via equation (4):

\[ P = 1 - \eta \]  

(4)

It is noted that for particulate filters with micron-size fibers, higher filtration efficiency results in higher air flow resistance.\textsuperscript{32,33} As suggested by US Department of Energy, the pressure drop of a HEPA filter should be less than about 325 Pa at a 4 L/min standard air flow.\textsuperscript{25} Also, it is suggested that the respirator filters should possess a pressure drop of less than 147 Pa which is very challenging for the high efficiency filter media.\textsuperscript{30} Thus, in order to correlate the pressure drop and removal efficiency for evaluating the overall performance and calculation of the benefit-to-cost ratio of a filter, quality factor (QF), that is, the figure of merit (FOM), needs to be considered.\textsuperscript{26–29} Some researchers utilize the specific filtration efficiency term recently in which the absolute filtration efficiency is normalized based on the weight of the filtering material.\textsuperscript{34}

FIGURE 1.3. Air filtration measurement setup.
1.1.2.2 Chemical Air Filters

In general, the gaseous chemical pollutants efficiency is defined based on the mass and/or concentration change of the pollutant upstream and downstream of the filter.\(^{16}\) However, due to the complexity and volatile nature of these pollutants, the removal efficiency of a filter is measured against a diluted and specific type of chemical as a function of time.\(^{16,35,36}\) The suspensions are then characterized utilizing various techniques such as ultraviolet (UV),\(^{16,37,38}\) liquid chromatography (HPLC),\(^{39}\) gas chromatography (GC),\(^{40}\) adsorption, specific gas sensors, etc.

Chemical air filters are commonly used to filter the gaseous pollutants from the air. They are usually made of traditional fibrous filter media which contain high loadings of activated carbon that can absorb small chemical molecules due to its large surface area. However, it is noted that this type of air filters are design for removal of gaseous pollutants and do not possess high particulate efficiency.

1.1.2.3 Antimicrobial Air Filters

Bioaerosols are one of the most hazardous pollutants threatening the human health; thus, a high-performance air filter is expected to not only capture the biohazards, but also to kill the biohazards. However, accumulation of the captured bioaerosols on air filters raises a critical issue as they can grow and spread on the filter in presence of sufficient moisture and nutrients.\(^{41-44}\)

Moreover, the particulate and chemical pollutants captured on the filter can further contribute to the growth of the bioaerosols which may lead to a significant decrease in filter’s efficiency and eventually deterioration of the filter (bioporation). Additionally, the volatile chemicals produced
from the microbial metabolism can also be released from the damaged filters to the air again.\textsuperscript{41,43} Antimicrobial air filters are designed to capture and terminate biological contaminants from the air. Conventionally, antimicrobial properties are realized by treating or mixing fibrous filters with different conventional antimicrobial agents such as heavy metals (e.g. silver nanoparticles),\textsuperscript{45,46} metal oxides (e.g. Titania (TiO$_2$), zinc oxide (ZnO) and copper oxide (CuO)),\textsuperscript{47,48} which are costly and can cause irreversible damages to the environment and human health themselves.

1.2 CAPTURING MECHANISMS OF AIR FILTERS

Mechanism of filtration is defined as an approach in which pollutants captured, attached and/or absorbed by the filtering media. Filtration mechanism of particulate and gaseous pollutants is mostly different. It is essentially dependent on the characteristics and properties of pollutants and filtering medium. Particulate filtration via fibrous media is controlled largely by physical parameters such as fiber diameter, pollutant size, flow rate and etc. while filtration of gaseous pollutants is mostly dominated by chemical mechanisms.

1.2.1 Particulate Capturing Mechanisms

The filtration function of particulate air filters is mainly realized via physical and PM size-based capturing mechanisms which determine the pollutant’s retention of the filter media. Sieving is one of the most important mechanisms and is only effective for particles with sizes larger than the pore size of the filter. However, the fibrous structure of the filter, either micro or nanoscale, is able to retain the particulate pollutants physically smaller than its pore size for which the sieving is ineffective. For particles with sizes smaller than the pore size of the filter, the filtration mechanisms include gravitation, straining, inertial impaction, interception, diffusion, electrostatic attraction,
and fiber-pollutant interaction on the surface of the filter. However, it is noted that for nanofibers, gravitation, straining and inertial impaction are less effective than interception and diffusion mechanisms. Figure 1.4 schematically shows the filtration mechanisms around a single fiber. Straining is an important filtering mechanism of HEPA and Ultra-low particulate air filters (ULPA) and only takes place when the particulate pollutants flow in the pathways between couple or more fibers with dimensions less than the particle aerodynamic diameter. Interception and diffusion are the most important filtration mechanisms of nanofibrous filters. Interception occurs when small particles flow with the air stream and their distance with the fiber surface is equal or less than their radius. This mechanism is predominant for capturing particles with 0.5 μm. The diffusion mechanism is effective for even smaller particles with an aerodynamic size smaller than 100 nm. For these particles, the movement is dominated by Brownian motion and capturing occurs via random collision. Studies show that particles with sizes in the range of 50 to 500 nm are removed by the nanofibrous media due interception and Brownian diffusion.

In fact, the effectiveness of these mechanisms for nanofibers is highly dependent on the fiber diameter and nanofiber packing density (mass of nanofibers per unit length which is a function of fiber density and filter thickness) as well as the particle’s Peclet and Knudsen number. Studies show that smaller fiber diameter and higher packing density nanofibers result in the enhancement in interception effect. Moreover, the Knudsen number is the ratio between the free pathway of the air stream and the particle size. In addition, the Peclet number signifies the strength between interception and diffusion mechanisms. A larger Knudsen number and a smaller Peclet number is critical for strong diffusion mechanism to occur.
1.2.2 Gaseous Chemicals Capturing Mechanisms

Gaseous pollutants are very small molecules and cannot be filtered efficiently via physical and size-based mechanisms introduced earlier. Intermolecular interaction mechanisms such as physiosorption and chemisorption are the two primary capturing mechanisms of gaseous chemical pollutants. Fiber-pollutant molecular interactions such as Van der Waals interaction, polar-polar interactions, hydrogen bonding and etc. occur at the fiber surface results in the physiosorption capturing of the pollutants. Nanofabric filters possess much higher surface area which increases the possibility of physiosorption. Therefore, nanofibers are more appropriate for filtration of gaseous chemicals. On the other hand, chemisorption occurs when the pollutants chemically bond to the surface of the fibers via either a catalytic or non-catalytic chemical reaction.
which leads to the conversion of pollutants into simpler compounds. The existence of active functional groups in the structure of nanofibers can significantly increase the likelihood of chemisorption.\textsuperscript{24} Therefore, electrospinning of the functional nanofibers can be a useful approach for filtration of gaseous chemical pollutants.

\subsection*{1.2.3 Mechanisms of Antimicrobial Function}

Biological contaminants, bioaerosols, are particulate pollutants with various sizes that can be captured by a fibrous filtering media via the physical and size-based mechanisms introduced earlier. In order to inactivate and kill the captured biological contaminants and reduce the living microorganisms detached from the filter when the air passes through, conventionally, antimicrobial properties are provided by treating fibrous filters with different conventional antimicrobial agents such as heavy metals (e.g. silver nanoparticles),\textsuperscript{45,46} metal oxides (e.g. Titania (TiO\textsubscript{2}), zinc oxide (ZnO) and copper oxide (CuO)),\textsuperscript{47,48} Numerous researchers suggested that the interactions between the positively charged nanoparticles and the negatively charged bacterial cells, particularly thiol (sulfhydryl) groups existing in amino acids such as cysteine,\textsuperscript{54–56} is the key factor for biocidal activities. In particular silver nanoparticles, the most commonly used antibacterial agent, attack the thiol groups in enzymes and proteins of bacteria causes the release of K\textsuperscript{+} ions from bacteria. Then silver ions were deposited in the vacuole and cell wall as granules and can lead to marked inhibition of bacteria growth.\textsuperscript{54} This phenomenon damages the cell envelope increases the bacteria size; thus, changes the structure of the cytoplasmic membrane, cytoplasmic composition, and outer cell layers. Silver ions can eventually interact with nucleic acids and DNA and cause the death of bacteria.\textsuperscript{54,57} It is noted that although the biocidal effect of
microorganisms is well known, the significance of the mechanism in terms of fatal action is still not clearly understood.\textsuperscript{46,54,58,59}

\subsection*{1.3 ELECTROSPINNING PROCESSING AND POLYMERIC NANOFIBERS}

Nanofibers are fibers with nanometer scale diameters which can be produced from various polymers, both synthetic and natural, with different properties for numerous applications. The diameter of nanofibers is highly dependent on the polymer type and production technique.\textsuperscript{60} Nanofibers can be generated by various methods, including self-assembly,\textsuperscript{61,62} template synthesis,\textsuperscript{63,64} drawing,\textsuperscript{65,66} thermal-induced phase separation,\textsuperscript{67} and electrospinning.\textsuperscript{68–71} All polymeric nanofibers possess very large surface area-to-volume ratio, noticeable mechanical properties, high porosity, flexibility and etc. in comparison with the conventional microfibers.\textsuperscript{72,73} Owing to these properties, nanofibers have been of great interest recently for many applications such as tissue engineering,\textsuperscript{74,75} wound dressing,\textsuperscript{76,77} drug delivery,\textsuperscript{78–80} lithium-air battery,\textsuperscript{81,82} and air filtration.\textsuperscript{27,28,71,83,84}

\subsubsection{1.3.1 Electrospinning}

Among all the nanofiber fabrication techniques, electrospinning is the most widely used method for production of nanofibers because of its facile setup and ability for mass production of nanofibers from numerous polymers. Although electrospinning is a relatively well-known fabricating technique, currently, it is the most advanced processing method to manufacture high performance nanofibers with high aspect ratio, specific surface-to-volume ratio, and high pore interconnectivity.\textsuperscript{73} Electrospinning was patented for the first time by A. Formhals in 1934.\textsuperscript{41}
Furthermore, Taylor developed a mathematical model to explain the effects of electric field on the formation of cone from a fluid droplet, also known as Taylor cone.\textsuperscript{85} Electrospinning and electrospraying history since 1745 to the recent day have been summarized by many researchers.\textsuperscript{60,86–89}

1.3.1.1 Process

Electrospinning is a simple but effective method for making uniform fibers ranging from micron and submicron fibers to, more significantly, to ultrathin fibers with various nanofiber structure and orientation via applying high voltage electric field.\textsuperscript{70,90,91} In typical electrospinning process, a viscoelastic fluid is loaded in a capillary tube mounted on a syringe pump. The fluid can be either a polymer solution or molten polymer. The polymer fluid then passes through a needle (nozzle) and a high voltage electric field is applied to the fluid. At the point that the electrically charged fluid overcomes its surface tension, the Taylor cone is formed at the tip of the nozzle. The fluid elongated toward the grounded collector electrode until the jetting occurs from the surface of the droplet. The jet experiences instabilities and fluctuates, which along with the fluid properties determine the morphology of the forming fibers. The simultaneous jet stretching and solvent evaporation during jet travel lead to the formation of nanofibers with various diameters and shapes. Figure 1.5 shows a schematic illustration of the electrospinning process.\textsuperscript{92–95} Fluid properties such as viscosity, polymer concentration, surface tension, conductivity, molecular weight, solvent system, etc. together with operating parameters including voltage, feeding flow rate, needle-to-collector distance, etc. play very important roles in the electrospinning process and properties of nanofibers. Electrospun nanofibers have tremendous potential in applications such as clean energy, health and environment areas, and air filtration.
1.3.1.2 Advantages for Air Filtration

Air filtration was the first commercial application of electrospun nanofibers at the early 1940s including Petrynov respiratory filters. This respiratory filter was effective for removal of PM pollutants; however, it was reported ineffective for filtration of gaseous chemicals. Electrospun nanofibers are particularly suitable for air filtration applications owing to their large specific surface area (approximately 1000 times higher than microfibers), extremely high surface energy (activity), and small pore size (about 4-100 times smaller than that of microfiber media). Electrospun nanofiber membranes are fit to prepare antimicrobial air filters, filter media for HEPA, respiratory protection, etc. either solely or alongside with conventional air filtering media. The role of the nanofiber layer in filtration of pollutants is highly dependent on its configuration.
(either positioned before or after microfibers). Thereby, incorporation of a very thin layer of spun nanofibers can significantly enhance air filtration performance.

1.3.2 Polymeric Nanofibers

A wide variety of synthetic polymers and biopolymers with or without incorporation of additional additives are applicable for fabrication of nanofiber membranes via electrospinning technique both in research laboratories and in practice. Fluid characterizations such as polymer viscosity, conductivity, surface tension, etc. are governed by polymer concentration, molecular weight and distribution, solvent, etc. Thus, the electrospinning parameters must be adjusted based on the fluid characterizations and can vary from polymer to polymer. Examples of synthetic polymers include polyethylene (PE), polypropylene (PP), polystyrene (PS), polyacrylonitrile (PAN), polycaprolactone (PCL), polyurethane (PU), etc.\(^{72,101–105}\) Moreover, cellulose, collagen, gelatin, keratin, zein, chitosan, etc. are some examples of natural biopolymers used for production of nanofibers.\(^{27,28,46,72,106–108}\)

1.3.2.1 Synthetic Polymers

Synthetic polymers are the most commonly used raw materials in electrospinning to produce air filtering media. The reason is that synthetic polymers have more stable chemical and thermal properties which result in more controllable fluid parameters and more facile for electrospinning.\(^{24}\) Polyethylene (PE), polypropylene (PP), polyacrylonitrile (PAN), and polyamide (PA) are often used to produce air filters. PA polymer possesses short di-amine and di-acid which makes the electrospinning very easy. PA nanofibers have been used for air filtration due to their small fiber diameter, large surface area, and high electrostatic charge.\(^{70,109}\) PA nanofiber filters show five
times reduced areal density and about three times lower air flow resistance compared with that of the commercial glass fiber filters.\textsuperscript{110}

PAN nanofibers have been recently studied for air filtration applications.\textsuperscript{71} It is reported that the PAN nanofilters possess more than 95\% efficiency for removal of PM\textsubscript{2.5} pollutants under hazardous air condition (PM\textsubscript{2.5} index $> 300$) during the field test. Due to the high dipole moment of PAN, it was able to capture particulate pollutants almost over 5 times its own fiber mass and high single fiber removal capability (Figure 1.6).

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{image1}
\caption{SEM image showing the detailed morphology of single fiber PM capturing capability. Scale bar, 1 μm.\textsuperscript{71}}
\end{figure}

\subsection*{1.3.2.2 Natural Biopolymers}

Natural materials gained more attention as nanofiber matrices for air filtration applications, recently. Table 1.1 introduced some of the common biomaterials such as keratin and chitosan that have been studied for this purpose. Limited number of natural materials nanofibers have been electrospun for air filtration applications because of the structural complexity of these materials which makes the nanofiber fabrication extremely difficult and challenging. Therefore, natural materials are blended with various conventional polymers such as PA, PVA, PEO, etc. to fabricate
nanofiber films. For example, chitosan has been mixed with poly(ethylene oxide) and fabricated into nanofibers as a filter material. The cationic nature of chitosan was used to achieve more than 70% removal of heavy metal ions in addition to its good antimicrobial properties.\textsuperscript{111} Also, keratin was blended with PA polymer and fabricated into nanofibers with giber diameter of about 150 nm. It is reported that the electrostatic nature of keratin microfiber filters can remove PM pollutants via columbic attraction.\textsuperscript{112} Moreover, it can absorb some toxic chemicals including VOCs due to its molecular structure. However, no results are presented about the use if keratin nanofiber filters for air filtration applications.\textsuperscript{112}

Natural proteins, are one of the most important “green” polymers. In particular, they possess a chemical structure uniqueness (e.g. rich functional groups), are abundant in nature, and biodegradable and have an economic viability. Engineering new air filtering products made of these raw materials can significantly decrease our reliance on petroleum, benefits the environment, and promote the economy. Therefore, in this dissertation, development of new generation of bio-based air filters will be presented. This research will be significant for promoting safe disposal and protecting the environment, refining the sustainability of agricultural systems, and benefiting the economy.

\textbf{1.3.2.3 Composite Materials}

Nanofiber filtering membranes of conventional synthetic polymer can be functionalized through specific additives to enhance the filtration performance of the air filters. For example, a carbon nanotube (CNT)-based composite filter mat was studied, recently.\textsuperscript{113} They used a combination of multiple layers of aligned CNT sheets, produced via an expensive chemical vapor deposition (CVD) method, with a PP filter mat to improve the filtration performance to 99\% efficient for capturing PM\textsubscript{0.3} particles. Although this study presented high particulate removal efficiency, it is
almost incapable of removing any toxic chemicals from the air due to the lack of functional groups in their filtering material structure. Moreover, in order to remove toxic chemicals, such as formaldehyde (HCHO), hexanal, and VOCs, researchers recently have focused on photocatalytic and thermal catalytic filtration using metal oxide catalyst nanoparticles, such as manganese oxide, copper oxide, gold/zirconium oxide (Au/ZrO₂). For example, effects of poly(ethylene terephthalate) (PET) fibers coated with Au/ZrO₂ catalyst nanoparticles on filtration of HCHO and CO gases were investigated and conversion to CO₂ and H₂O with rates of 83% and 90%, respectively was reported after 20 minutes and decreasing over time. This study showed that Au/ZrO₂/PET air filters have very high CO and HCHO removal efficiencies. Table 1.1 summarize the polymers used for fabrication of nanofibers for different applications in details.

**TABLE 1.1.** Synthetic and natural polymers utilized for generation of nanofibers for different applications

<table>
<thead>
<tr>
<th><strong>Polymeric materials</strong></th>
<th><strong>Fabrication method/application</strong></th>
<th><strong>References</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Commonly used conventional plastics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polypropylene (PP)</td>
<td>Melt electrospinning. Filtration</td>
<td>102,103</td>
</tr>
<tr>
<td>Polyethylene (PE)</td>
<td>Melt/solution electrospinning. Filtration</td>
<td>102,115</td>
</tr>
<tr>
<td>Poly(vinyl chloride) (PVC)</td>
<td>Electrospinning. Filtration</td>
<td>46,116</td>
</tr>
<tr>
<td>Polystyrene (PS)</td>
<td>Electrospinning. Filtration, matrix for composite materials</td>
<td>117,118</td>
</tr>
</tbody>
</table>

**Advanced and special polymers**
<table>
<thead>
<tr>
<th>Material</th>
<th>Applications</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyacrylonitrile (PAN)</td>
<td>Electrospinning. Filtration, precursor for CNF fabrication, etc.</td>
<td>71,119–121</td>
</tr>
<tr>
<td>Polycaprolactone (PCL)</td>
<td>Electrospinning. Tissue engineering, wound dressing, etc.</td>
<td>122–124</td>
</tr>
<tr>
<td>Poly(ethylene oxide) (PEO)</td>
<td>Electrospinning. Model material, filtration</td>
<td>125,126</td>
</tr>
<tr>
<td>Poly(vinyl alcohol) (PVA)</td>
<td>Electrospinning. Food packaging, carrier for conductive polymers, filtration, etc.</td>
<td>95,105,127,128</td>
</tr>
<tr>
<td>Polyurethane (PU)</td>
<td>Electrospinning. Protective clothing, matrix for composite materials, filtration, etc.</td>
<td>104,129</td>
</tr>
<tr>
<td>Polyamides (PA)</td>
<td>Electrospinning. Filtration, reinforcement, model material, etc.</td>
<td>130–133</td>
</tr>
<tr>
<td>Poly(methyl methacrylate) (PMMA)</td>
<td>Electrospinning. Removable core, matrix for composite materials, etc.</td>
<td>134–136</td>
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</tbody>
</table>

**Natural biomaterials**

<table>
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<tr>
<th>Material</th>
<th>Applications</th>
<th>Pages</th>
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<tbody>
<tr>
<td>Collagen and gelatin</td>
<td>Electrospinning. Biomedical applications, packaging, filtration, etc.</td>
<td>28,137,138</td>
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<tr>
<td>Cellulose and lignin</td>
<td>Electrospinning. Biomedical applications, precursor for CNF, filtration, etc.</td>
<td>139–141</td>
</tr>
<tr>
<td>Chitosan</td>
<td>Electrospinning. Biomedical applications, antimicrobial, filtration, etc.</td>
<td>111,142</td>
</tr>
</tbody>
</table>
1.4 NANOFIBERS CHARACTERISTICS FOR AIR FILTRATION APPLICATION

Physical characteristics of the nanofiber filter including fiber diameter and distribution, pore size and distribution, specific surface area, areal density, and thickness can significantly tune the air filtration performance of an air filter. In addition to the physical characteristics of the filter, polymer chemical characteristics such as structural functional groups play very important role in tailoring the air filtration properties, in particular, chemical removal efficiency, of the nanofiber membrane.

1.4.1 Physical Characteristics

1.4.1.1 Fiber Diameter and Pore Size

Fiber diameter and pore size play very important roles in filtration performance of a filter membrane. It is reported that for removal of particles with sizes up to 0.3 μm with efficiency above 99%, nanofiber filters with a fiber diameter of less than 300 nm is most suitable. A study on PAN filter demonstrated that a decrease in fiber diameter from 1μm to 200 nm resulted in a significant increase in particulate efficiency from 48.21 to 98.11%. Smaller particulate pollutants diffuse easily into higher fiber diameters and interception is dominated over diffusion. The small fiber diameter reduces the pore size and enhances the diffusion and direct-interception for the smaller particles. The fiber diameter distribution is also very important and influences the filtration
performance of the filter media. A unimodal distribution causes lower air flow resistance and normalized thickness compared with that of a wider or bimodal distribution with the same average fiber diameter.\textsuperscript{144} Although the smaller fiber diameter increases the filtration efficiency, it can cause an increase in pressure drop as well.\textsuperscript{53} However, recently, it is discovered that the smaller fiber diameters (less than 100 nm) can provide improved aerodynamic slip where the collision between air molecules and nanofibers are almost eliminated. Therefore, this slip flow decreases the friction and the pressure drop does not elevate which was reported before.\textsuperscript{145,146}

In addition to the fiber diameter, nanofabric pore size also plays a critical role in filtration properties, particularly the pressure drop of the filter. In general, nanofibers can provide three different types of pores: (1) closed pores, which are not reachable; (2) blind pores, which are terminated inside the structure of the media; and (3) through pores, which are open and effects the filtration performance.\textsuperscript{147} The smaller pore size results in a very high filtration efficiency but unfavorably higher air flow resistance and large pores have an opposite effect. Therefore, the pore size can influence the pressure drop intensely. It is reported recently that the gradient of different pore sizes can enhance the filtration performance of filtering media without largely increasing the pressure drop of the filter.\textsuperscript{93} This is because of created circulatory flow between large and small pores and more pathways for the air molecules to flow.\textsuperscript{93} Moreover, the pore size distribution is also vital to improve the filtration performance of the filter media.

\subsection*{1.4.1.2 Fiber Areal Density and Thickness}

Fiber areal density is defined as the mass of the nanofibers per unit area. The thickness of a nanofiber filter directly depends on the areal density of the nanofibers which linearly depends on the amount of the electrospun solution and electrospinning time.\textsuperscript{148} Higher area density of
nanofibers and thicker filter membrane lead to achieving high filtration efficiency; however, it adversely causes a great increase in the pressure drop of the filter.\textsuperscript{149} It is noted that increasing areal density and thickness of nanofiber layer does not enhance the filtration efficiency beyond a certain limit, but continues to increase the air flow resistance and, as a result, fading the quality factor of the filter. Recent studies show that the filtration performance can be boosted if the nanofibers with low areal density and thickness are stacked in layers compared with one thick layer of nanofabrics. However, no study shows a precise areal density or thickness to achieve high filtration performance and low pressure drop; thus, optimization of areal density and thickness have to be done for specific application to achieve a filter with a high quality factor.

1.4.2 Chemical Characteristics

Physical characteristics of nanofiber media control the filtration properties for removing the particulate pollutants; however, they are not sufficient for capturing gaseous chemical molecules. Polymer’s chemical characteristics such as structural functional groups play a very important role in air filtration properties for gaseous chemicals. As it was discussed above, the commercial air filters are made of conventional polymers such as PP, PE and glass microfibers. The filtration function of these air filters is mainly realized via physical and PM size-based capturing mechanisms. Thus, because of lack of active functional groups in the structure of these materials, no interactions exists between fiber and pollutants. In order to address this issue, recently researchers focus on polymers with polar functional groups such as polyacrylonitrile (PAN), polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), polyamide (PA), and polyimide (PI).\textsuperscript{71,150,151} These materials have active functional groups with positive dipole moment that can interact and capture the pollutants. Therefore, nanofabrics of polymers rich in functional groups
represent a promising solution for high performance air filtering materials. In particular, biomaterials, such as natural proteins, are promising candidates as high-performance air filtering materials. It is well-known that proteins are rich in functional groups, that is, the R-groups on the amino acids. These functional groups make proteins an ideal material for air filtering application. Therefore, in this dissertation, we developed protein-based nanofiber filters with high filtration efficiency for both particulate and gaseous pollutants.

1.5 AIR FILTRATION PERFORMANCE OF NANOFABRIC MEMBRANES

This section is aimed to compare the air filtration performance of various nanofiber membranes recently studied. In order to assure that the results are comparable, studies were chosen that have similar air filtration test conditions.

1.5.1 Particulate Filtration Performance

Recently, most of the studies have focused on developing a nanomembrane filter with enhanced particulate removal efficiency. Here, the particulate filtration performance of numerous polymeric nanofilters for PM pollutants with different sizes are compared and Table 1.2 represents the summary of these studies. In most of these studies, the testing air face velocity of about 5 cm/s was chosen. It is reported that increasing the air face velocity to about 15 cm/s results in a dramatic rise of pressure drop. It is noted that maintaining low pressure drop values at higher air face velocities is one of the challenging issues for nanofilters. It can be found from Table 1.1 that PLA biodegradable polymer led to the highest filtration efficiency for 0.3 μm particles. Moreover, these
results indicate that filtration performance of the polymer blends (e.g. PA/PAN) is better than their counterpart pristine nanofilter.

TABLE 1.2. Particulate filtration performance of different nanofilters.

<table>
<thead>
<tr>
<th>Material</th>
<th>Areal density (g/m²)</th>
<th>Fiber diameter (nm)</th>
<th>PM size (μm)</th>
<th>Removal efficiency (%)</th>
<th>Pressure drop (Pa)</th>
<th>Quality factor (Pa⁻¹)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAN</td>
<td>NA</td>
<td>200</td>
<td>&lt;0.1</td>
<td>96.12</td>
<td>133</td>
<td>0.024</td>
<td>71</td>
</tr>
<tr>
<td>PI</td>
<td>NA</td>
<td>300</td>
<td>&lt;2.5</td>
<td>97.02</td>
<td>45</td>
<td>0.078</td>
<td>151</td>
</tr>
<tr>
<td>PA</td>
<td>1.5</td>
<td>150</td>
<td>0.3</td>
<td>99.5</td>
<td>500</td>
<td>0.012</td>
<td>143</td>
</tr>
<tr>
<td>PU</td>
<td>0.7</td>
<td>120</td>
<td>&lt;0.4</td>
<td>99.66</td>
<td>150</td>
<td>0.028</td>
<td>93</td>
</tr>
<tr>
<td>PA/PAN</td>
<td>3</td>
<td>272</td>
<td>0.3</td>
<td>99.99</td>
<td>100</td>
<td>0.12</td>
<td>152</td>
</tr>
<tr>
<td>PAN/PU</td>
<td>24</td>
<td>175</td>
<td>0.3-0.5</td>
<td>99.98</td>
<td>120</td>
<td>0.068</td>
<td>153</td>
</tr>
<tr>
<td>PAN/SiO₂</td>
<td>2.1</td>
<td>650</td>
<td>0.3-0.5</td>
<td>99.98</td>
<td>117</td>
<td>0.07</td>
<td>69</td>
</tr>
<tr>
<td>PP/CNT</td>
<td>121/0.1</td>
<td>40</td>
<td>&gt;0.1</td>
<td>99.99</td>
<td>150</td>
<td>0.061</td>
<td>113</td>
</tr>
<tr>
<td>PEO/Chitosan</td>
<td>NA</td>
<td>100</td>
<td>0.26</td>
<td>70</td>
<td>NA</td>
<td>NA</td>
<td>111</td>
</tr>
<tr>
<td>PLA</td>
<td>5.2</td>
<td>225</td>
<td>0.3</td>
<td>99.997</td>
<td>170</td>
<td>0.065</td>
<td>154</td>
</tr>
<tr>
<td>PVC/PU</td>
<td>21</td>
<td>960</td>
<td>0.3-0.5</td>
<td>99.5</td>
<td>145</td>
<td>0.036</td>
<td>98</td>
</tr>
</tbody>
</table>

1.5.2 Gaseous Chemical Filtration Performance

Despite many studies on particulate air filters, a limited number of studies have been conducted on the high efficiency gaseous chemical filtering materials. Filtration of gaseous pollutants via
nanofiber membranes is still a complex and challenging issue. As describes above, gaseous chemical pollutants capturing mechanisms are governed by interaction-based mechanisms. It means that the gaseous pollutants can be absorbed, adsorbed or reduced by the nanofibers. Recently studies reported filtration of formaldehyde (HCHO), carbon monoxide (CO) and nitrogen oxides (NO$_x$) by chemical reduction/conversion and capturing VOCs by adsorption mechanisms via nanofilters.

**Reduction/Conversion of HCHO and CO:** In order to remove toxic chemicals, such as formaldehyde (HCHO), researchers recently have focused on photocatalytic and thermal catalytic filtration using metal oxide catalyst nanoparticles, such as manganese oxide, copper oxide, gold/zirconium oxide (Au/ZrO$_2$). For example, effects of poly(ethylene terephthalate) (PET) nanofibers coated with Au/ZrO$_2$ catalyst nanoparticles on filtration of HCHO and CO gases were investigated and conversion to CO$_2$ and H$_2$O with rates of 83% and 90%, respectively was reported after 20 minutes and decreasing over time. This study showed that Au/ZrO$_2$/PET air filters have very high CO and HCHO removal efficiencies.$^{114}$ However, it is noted that the nanoscale raw materials and deposition methods are complicated and very costly. Additionally, it is only effective for the removal of CO and HCHO due to the selective catalysts and nanoparticle types, therefore, it requires different catalysts and metal oxides to capture more species or complex gaseous chemicals in the polluted air.

**NO$_x$ reduction:** Recently, studies reported the removal of NO$_x$ pollutants via nanofabric filter without producing any harmful byproducts.$^{155}$ For instance, porous carbon fiber (CNF) filter produced from oxidation and carbonization of PAN nanofabrics was studied for NO filtration at
room temperature. The study shows that CNF media first adsorb NO molecules and then the NO molecules undergoes a catalytic oxidation and convert to NO$_2$ and particularly reduction of N to N$_2$.$^{38}$ In another study, Tin oxide/chromium oxide (SnO/CrO : 95/5) was added to PU nanofibers with 3% concentration to filter NO$_x$ and CO$_x$. The results show that these metal oxides converted NO$_x$, CO and CO$_2$ with 80% catalytic efficiency.$^{156}$

**VOC removal:** Many studies have focused on filtration of VOCs such as acetone,$^{37,157}$ toluene, chloroform,$^{157}$ and benzene$^{40}$ via the addition of metal oxide and biopolymers to electrospun nanofibers of synthetic polymers. For example, titania nanoparticles (TiO$_2$) was added into PVA nanofibers to filter acetone molecules. The results showed that at a lower concentration of acetone (about 250 ppm) the PVA/TiO$_2$ nanofilters was able to reduce 98% of acetone pollutants in 10 seconds. On the other hand, in a high concentration of acetone (>1500 ppm) the photocatalytic activity of TiO$_2$ has reduced quickly and the removal efficiency decreased to 54%.$^{37}$ In another study, PU nanofibers are reported with the capability to adsorb toluene, chloroform and hexane VOCs. They reported that the filtration efficiency of these VOCs improved to almost 2 times by incorporation of fly ash particles (FAP). The VOCs are adsorbed on the surface of the nanofibers by $\pi$-complex bonding.$^{37,157}$

1.6 DISADVANTAGES AND CHALLENGING ISSUES OF AIR FILTERING MATERIALS

As described above, currently there are three main types of air filters: particulate air filters (e.g. HEPA), chemical air filters (e.g. activated carbon), and antimicrobial air filters (e.g. silver particles). Traditionally, air filters are made of porous films, such as non-woven fibrous mats with
randomly oriented micro-size fibers. The fibers are made of chemically synthesized or petroleum-based materials, such as polypropylene and fiberglass. Antimicrobial air filters usually include high loadings of expensive nanoparticles, such as silver or titanium oxide that provide their antibacterial properties. However, PM and gas filters give negligible protection against each other’s hazard. Therefore, in current air filtering systems, in order to provide a high quality air downstream of the air filter, many different types of air filters such as regular particulate, HEPA, activated carbon, and antimicrobial, among others, are needed at the same time. For these systems, there are issues relevant to their pressure drop downstream of the filters and, as a result, energy consumption and cost for active air exchange. Disadvantages associated with current air filtering materials used in commercial air filters and being studied in research labs are summarized below:

(1) **Materials:** None of the synthesized materials (e.g. PP), glass fiber and various additives are naturally disposable or environmentally friendly. In other words, used air filters can be a source of secondary environmental pollution.

(2) **Functionality:** Limited filtrations of various pollutants, because of (a) insufficient interactions with toxic pollutants due to lack of functional groups in the filtering materials structures; (b) extra particles (e.g. activated carbon or nanoparticles) loading into the filter mats may reduce particulate filtration efficiency and/or worsen the pressure drop performance, which will increase the energy consumption for active air exchange.

(3) **Processing:** Fabrication of the functional air filtering materials involves various complicated and costly processes, especially those chemical and antimicrobial air filters which require selected catalysts and nanoparticles.

(4) **Filtration mechanisms:** Current filtration process is governed by physical and size-based mechanisms that are only efficient for capturing particulates. These filtration mechanisms
are inadequate for the removal of toxic gaseous chemicals (usually various polar molecules) and antimicrobial filtering.

Therefore, the development of cost-effective bio-based multi-functional air filtering materials with high removal efficiencies for various types of pollutants (e.g. particles, toxic chemicals, biological hazards, etc.) while maintaining low air flow resistance represents a challenging task for the development of advanced bio-based materials for air filtration systems.

1.7 OBJECTIVES AND SIGNIFICANCE

The objective of this dissertation is to create high-efficiency protein-based multi-functional air filtering materials. These novel air filtering materials will be able to simultaneously remove various airborne pollutants such as particulate matters (e.g. those with the diameter range of 0.1 – 10 µm), toxic chemical gases (e.g. formaldehyde, carbon monoxide, sulfur dioxide, and volatile organic compounds), and biological hazards (e.g. bacteria and viruses) with high efficiencies. In addition, they are cost-effective, light weight, environmentally friendly and their disposing process will not cause secondary environmental issues while possessing very low air flow resistance.

Commercial High Efficiency Particulate Air filters (HEPA) in the market are considered to possess the highest removal efficiency for the particles with different sizes, in particular, the particles with 0.3 µm size. However, these HEPA filters can hardly remove toxic chemicals (e.g. gas molecules) from the air. Those functional air filtering materials in the market or being studied don’t have such high particulate efficiency filtration. For example, chemical air filters contain huge amount of activated carbon or metal oxide catalysts which possess low filtration efficiency for particulate pollutants. Special anti-microbial air filters in the market contain a high loadings of silver
nanoparticles to provide the antimicrobial property, but they are costly materials and hard to naturally decompose. In order to remove those hazardous particles, toxic chemicals, and biohazards from the air, different types of air filters need to be utilized at the same time. As a consequence, developing “green” air filtering materials with high efficiencies for simultaneous removal of particulate and chemical is becoming an urgent need and challenging task. This dissertation aims to create protein-based air filtering materials by electrospinning processing technique.

The specific research objectives are:

1) To fabricate protein-based air filtering materials via study of the processing and establish the relationship between processing parameters and the structures of the resulting nanomaterials for achieving optimized materials. Two example materials will be studied:
   (A) Pure gelatin protein, air filtering material: study the effects of “green” solvent and the solvent composition on electrospinning processing and microstructure (fiber diameter and distribution) of the nanofabrics; study the hierarchical technique to achieve novel porous fiber morphology
   (B) Plant protein-based nanocomposite air filtering material: study the denaturation of soy protein, an abundant plant protein, and its effects on the microstructures/morphology (nanofiber diameter and uniformity)
   (C) Paper Towel/Protein hybrid air filters with gradient structures for capturing pollutants of broad species: study the role of paper towel in the enhancement of air filtration performance; study the environmental stability of the hybrid air filters.

2) To study the air filtration performance of the fabricated materials and establish the relationship between the filtering performance and the nanofiber microstructures:
Study the effects of nanofiber diameter and uniformity on air filtration performance (particulate filtration efficiency and air flow resistance); study the toxic chemical (e.g. CO, HCHO, SO₂, and VOCs) removal efficiency; determine the protein-based air filtering materials with optimized particulate and chemical removal efficiencies; study the antimicrobial property of the protein-based nanofabrics.

3) To investigate the new filtration mechanism: substantiate the relationship between the newly proposed filtering mechanism and pollutant capturing performance:
   Analyze the active functional sites available in the protein structure; study the interactions between those functional groups and pollutants; study the different stages of filtration process of the nanofiber mats.

The significance of this dissertation can be summarized as follows:

(1) For environment: Abundant natural proteins are selected to be used for fabrication of high-performance “green” air filtering materials. This dissertation presents two types of abundant proteins to be fabricated via facile electrospinning technique. The use of biomaterials which are environmentally friendly and cost-effective materials can avoid the secondary pollutions to the environment at their disposal after use.

(2) For new filtration capability: It is a breakthrough in generating extra filtration performance without relying on the addition of any other agents, such as capturing toxic (gas state) chemicals via the plenty of functional groups in the protein structures. As a key component in air cleansing systems, air filtering materials play an important role in removing pollutants, including both particulates and toxic chemicals, from the air. Previous studies on air filtration application as well as commercial air filters in the market, have simply
focused on removing only one or two types of pollutant. In this dissertation, abundant proteins with novel nanofiber morphologies are utilized to create multifunctional air filtering materials that are able to efficiently remove different types of pollutants simultaneously and combine various types of air filters into one single filtering mat without the addition of any active materials. This breakthrough will finally give rise to multifunctional air filtering materials which can be used in numerous air filtration applications.

(3) For new filtration mechanism: A new interaction-based filtration mechanism is proposed, which will be significant for the development of new air filtering systems. As it is mentioned above, numerous functional groups exist in protein structure. These functional groups can strongly interact with various pollutants in the air and enable the filter to remove the pollutants (toxic chemicals, solid particles, biohazards) via an interaction-based mechanism besides existing size-based primary mechanisms. This new mechanism enables the protein-based filter nanofabrics to not only possess extremely high particulate efficiency for particle matter with a broad range of size from 0.1 µm to 10 µm, but also, very high removal efficiencies for toxic chemicals and more significantly unexpected pollutants that might exist in the air due to photochemical reactions.
CHAPTER TWO: “GREEN” NANO-FILTERS: FINE NANOFIBERS OF NATURAL PROTEIN FOR HIGH EFFICIENCY FILTRATION OF PARTICULATE POLLUTANTS AND TOXIC GASES

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ABSTRACT: Particulate and chemical pollutants are ubiquitous in polluted air. However, current air filters using traditional polymers can only remove particles from the polluted air. To efficiently filter both particulates and chemical pollutants, development of multi-functional air filter materials with environmental friendliness is critically needed. In this chapter, gelatin is employed as an example to study the potential of natural proteins as high-performance air-filtering material. Based on an optimized composition of a “green” solvent, uniform gelatin nanofiber mats were fabricated via an electrospinning approach. For the first time, it is found that the resulting nanofabrics possess extremely high removal efficiencies for both particle matter (with a broad range of size from 0.3 μm to 10 μm) and various toxic chemicals (e.g. HCHO and CO). Moreover, these high efficiencies are realized by the protein nanofabrics with a much lower areal density (3.43 g/m²) when compared with that of commercial air filters (e.g. 164 g/m² for high efficiency particulate air filter (HEPA)). This chapter reveals that nanofabrics of natural proteins hold great potential for application in “green” and multi-functional air filtering materials.
2.1 INTRODUCTION

Air pollution has been of great concern due to the huge emission of particulate and chemical pollutions. The release of chemicals, particulates and biological materials into air can lead to various diseases or discomfort to humans and other living organisms, alongside other serious impacts on the environment. The unknown combination of particles and chemical pollutants makes the polluted air even more harmful. Particle Matter (PM) is usually categorized into two groups, PM$_{2.5}$ and PM$_{10-2.5}$ which denote particles with aerodynamic diameters smaller than 2.5 mm and between 2.5–10 mm, respectively.$^{3,4}$ According to the 2009 and 2012 World Bank report, more than 60% of Americans live in air quality levels that are potentially detrimental to health.$^{1,2}$ Recent studies have reported a more serious PM pollution problem in developing countries.$^6$ A high degree of air pollution was responsible for numerous premature deaths. PM$_{2.5}$ particles are the critical particulate pollution to be filtered due to their ability to penetrate into human lungs and bronchi.$^{3,4,6-11}$ Indoor air quality has become an increasing issue as well. More and more buildings incorporate air filtration protection in their heating, ventilation, and air conditioning systems, but a significant amount of energy is required to maintain the air exchange process due to a high air-resistance (pressure drop) of the air filters. Therefore, air filters with high-efficiency of removing particles and chemicals simultaneously are critically needed.

Understanding of the composition of polluted-air is critical for the development of air filtering materials. In general, the composition of pollutants in polluted-air is extremely complicated due to the complexity of the sources of pollution. PM particles can be produced from variety of sources, such as fuel combustion in vehicles, industrial factory plants, cigarette smoke, dust, etc. These PM particles behave distinctly due to their diverse chemical composition. Most PM$_{2.5}$ particles are
composed of organic compounds such as carbon derived matters (e.g. carbon dioxide and carbon monoxide), inorganic compounds (e.g. sulfur dioxide ($\text{SO}_2^2$), sulfate ($\text{SO}_4^{2-}$), silicon dioxide ($\text{SiO}_2$), and nitrate ($\text{NO}_3^-$), etc.), and biological threats (e.g. bacteria and viruses). These particles are very stable in air and have lifetimes between hours to weeks due to their very small sizes. They can scatter visible light and reduce visibility because of the similarity between their particle size and visible light wavelengths. In addition to PM particles, polluted air includes a wide variety of chemical gases such as carbon monoxide (CO), nitrogen dioxide ($\text{NO}_2$), methane ($\text{CH}_4$), benzene, dioxin, ozone, etc. A large number of chemicals in polluted air are classified as volatile organic compounds (VOCs) which are primarily emitted by petrochemical and allied industries. VOCs can undergo different kinds of photochemical reactions in the atmosphere and cause various environmental hazards. In gas phase carcinogenic or otherwise toxic VOCs present a danger to humans. Since the polluted air is usually composed of pollutants with complicated compositions and physicochemical properties, multi-functional air filtering materials that are able to generate various types of interactions with the pollutions are of great interest for air-filtering applications.

Air filters are the most common used device to remove pollutants from the air. They have been widely used in different areas, e.g. automotive industries, residential, general commercial, and even hospitals, general surgeries and so on. The filtration function is mainly realized via physical and PM size-based capturing mechanisms. There are four primary mechanisms for filtration based on the size of the pollutant particles. Sieving is one of the most important mechanisms and is only effective for particles with sizes larger than the pore size of the filter. For particles with sizes smaller than the pore size of the filter, inertial impaction, interception, and diffusion are the
dominant mechanisms for filtration. In specific, interception occurs when small particles flow with the air stream and come into contact with the fiber surface. The attractive interactions between the small particles and fibers play a critical role for this mechanism. The diffusion mechanism is effective for even smaller particles with aerodynamic size smaller than 100 nm. For these particles, movement is dominated by Brownian motion and capturing occurs via random collision. Traditionally, air filters are made of porous films, such as non-woven fibrous mats with randomly oriented micron-size fibers. These types of air-filtering materials have several disadvantages as explained below. First, the fibers are made of chemically synthesized or petroleum based materials, such as polypropylene and fiberglass. These conventional materials provide very limited chemical functionality, resulting in insufficient interactions with pollutants. Secondly, disposing of used air filters made of these materials can cause further environmental pollution as most of them are not environmentally friendly. Finally, microfiber-based air filtering materials possess limited surface area, which further deteriorate the filtration performance.

To address the above issues related to conventional air-filter materials, nanofiber mats have been of great interest recently. Nanofiber mats possess several advantages as explained below. Firstly, nanofibers will tend to absorb substance from the environment due to a high surface energy, which enhances the interactions between fibers and pollutants. Secondly, nanofibers can significantly increase the surface area of filter materials. In other words, nanofibers provide more active sites for trapping pollutants. As a result, nanofiber mats can realize a high filtering efficiency for PM while possess low pressure drop or air resistance, which is critical for their practical application. Therefore, nanofabrics of polymers rich in functional groups represent a promising solution for high-performance air-filtering materials. In particular, biomaterials, such
as natural proteins, are promising candidates as high-performance air filtering materials.\textsuperscript{27} It is well-known that proteins are rich in functional groups, that is, the R-groups on the amino acids. These functional groups make proteins an ideal material for air filtering application. For example, chitosan has been mixed with poly(ethylene oxide) and fabricated into nanofibers as a filter material. The cationic nature of chitosan was used to achieve more than 70\% removal of heavy metal ions.\textsuperscript{111} Other biomaterials were also studied as air filter materials,\textsuperscript{160} however, they were usually mixed with conventional polymers to fabricate nanofibers. As a result, the potential of pure protein nanofabrics as high-performance air filtering materials has never been studied based on the author's knowledge. Of course there are still challenging issues for scaling—up the process using metric tons of natural protein materials.

In this chapter, the potential of pure protein nanofabrics for air filtering application is investigated. It is believed that the combination of nanomaterials with natural proteins can lead to a powerful nanofabric with the ability to trap various kinds of pollutants, including particulate and toxic gas. In particular, gelatin is employed as an example for that. Gelatin protein is derived from thermal denaturation of collagen, the most abundant protein in human and animal bodies.\textsuperscript{161} Fabrication of gelatin nanofibers has been proved very successful and they are usually reported as scaffolds for food, energy, pharmaceutical, environmental, and medical applications,\textsuperscript{162–164} except as air filtering material. Here, to study gelatin nanofabrics for air filtration purposes, the fabrication of gelatin nanofibers is further improved. Firstly, instead of using toxic solvents (e.g. 2,2,2-trifluoroethanol (TFE) or 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP)) which are usually used for the electrospinning of gelatin,\textsuperscript{165} a non-toxic solvent (mixture of acetic acid and water) is
employed. Secondly, the diameter of the gelatin nanofibers is further reduced to be around 70 nm, which is smaller than the typical values (ca. 100 nm) for gelatin nanofibers.\textsuperscript{166–168}

2.2 EXPERIMENTAL

2.2.1 Raw Materials and Solution Preparation

Gelatin powder (type A) produced from porcine skin was supplied from Sigma-Aldrich (MO, USA). Acetic acid (99.9% purity) was purchased from J.T.Baker\textsuperscript{®} (PA, USA). Gelatin was dissolved in mixed solvent (volume ratio, acetic acid : DI water = 80 : 20) with a concentration of 18 wt% at 65 °C. The mixed solvent was used to achieve a good electrospinning of the gelatin solution. With that ratio between water and acetic acid, it was found that a homogenous yellow solution and stable electrospinning of the solution can be achieved.

2.2.2 Preparation of Protein Filter Nanaofabrics

Protein nanofabrics were prepared by electrospinning techniques. The gelatin solution was loaded in a syringe (Monojet\textsuperscript{TM} Kendall) with a 21-gauge blunt-tip needle. An operating voltage of 18–20 kV was employed for the electrospinning and was controlled by a high voltage power source (ES50P-5W, Gamma High Voltage Research). A mono-inject syringe pump (KD Scientific, KDS-100) was utilized to pump the gelatin solution. Commercial aluminum mesh with wire diameter of 0.011 inch and mesh size of 18 × 16 was grounded to collect the gelatin fibers. The distance between needle and sample collector was fixed to be 10 cm and an average flow rate of 0.6 ml/h was utilized.\textsuperscript{86,168}
2.2.3 Polluted-Air Samples Preparation and Air Filtering Testing

Cigarette smoke was used as the source of pollution to prepare polluted-air samples. It has been estimated that cigarette smoke includes PM particles with a broad range of sizes (0.01 to 10 μm), and more than 7000 different chemicals, hundreds of which are toxic. The most dangerous chemicals of interest for filtration are carcinogenic, such as formaldehyde (HCHO), carbon monoxide (CO), ammonia (NH₃), hydrogen cyanide (HCN) and toxic metal ions (chromium (Cr³⁺, Cr⁶⁺), lead (Pb²⁺), cadmium (Cd²⁺)). Since the original polluted-air samples were so concentrated with PM and chemicals, they were diluted in a gas bag to a hazardous level which can be measured by the analyzer. The diluted polluted-air with detectable level of pollutions was used as the final polluted-air sample for air-filtering testing. Before the air-filtering testing, the initial concentrations of PM with different particle sizes (0.3–10 μm) and toxic chemicals (HCHO and CO) in the air samples were measured by a particle counter (CEM, DT-9881). To perform the air-filtering testing, the pressure difference of both sides of air filter was controlled and measured by a manometer (UEi, EM201-B) with a standard air flow velocity of 5 cm/s to investigate the air flow resistance of the air filter material. In all the measurements, a circular filter sample with diameter of 37 mm was placed in a home-made sample holder. The filtered air was collected by another clean gas bag which was vacuumed in advance (the digital image of experimental setup is shown in Figure S1.1, Appendix I). When the air-filtering testing ended at different filtering time, the concentrations of the PM and toxic chemicals inside the clean gas bag with filtered air were measured and recorded. Via the equation 1, one can determine the removing efficiency η.
2.2.4 Characterizations

To study how the particle pollutants were removed by the protein nanofabrics, SEM (FEI SEM Quanta 200F) was employed to investigate the morphology of the protein nanofabrics before and after air filtering testing. The samples were sputter-coated with 10 nm gold nanolayer in thickness using Technics Hummer V sputter coater. In order to study the possible interactions between the protein nanofabrics and pollutants, FTIR (Nicolet, Thermo Scientific) absorption spectra was employed. To distinguish the interactions between nanofibers and pollutants from the interactions inside the fabric or polluted-air themselves, the FTIR spectrum of three kinds of samples were recorded and compared. These samples include polluted-air, clean protein nanofabrics before and after filtration. All the measurement was repeated at least 3 times and good repeatability was found for these samples.

2.2.5 Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) multiple comparison method. The confidence interval was set to 95%. The differences among the data with a p-value < 0.05 were reflected to be statistically significant.

2.3 RESULTS AND DISCUSSION

2.3.1 Gelatin Nanofabrics/Nanofibers Mats

The target of this chapter is to study the potential of gelatin nanofabrics as high-performance air filtering material with two levels of air filtering functions: (1) removing particles, such as dust, pollen, with particle sizes in a broad range from 0.1 to 100 μm; (2) removing toxic or obnoxious
gases, such as formaldehyde and carbon monoxide in tobacco smoke. It is known that gelatin molecules possess a broad range of functional groups in their multi-level structures. The characteristics of these chemical structures provide capability for interaction with multiple species of polar molecules, which lead to a great potential to capture many chemicals. Specifically, gelatin consists of glycine (21.4%), proline (12.4%), hydroxyproline (11.9%), and glutamic acid (10.0%) in its amino acid profile.[35,36] The amino acids bring gelatin various functional groups (such as carboxylic and hydroxyl, charged groups, and many other polar/nonpolar functional groups). These functional groups can act as active sites generating numerous interactions with pollutants, including hydrogen bonding, ionic bonding, and charge–charge interactions and so on.170,171 Combined with electrospinning technique, gelatin nanofibers can be fabricated (Figure 2.1). Electrospinning is an effective method for making uniform nanofibers with high aspect ratio, and high pore interconnectivity with size ranging from micron to nanometer scale.
FIGURE 2.1. Schematics of gelatin solution preparation and nanofibers fabrication via electrospinning, followed by the schematics of a single gelatin nanofiber with a functional surface due to various functional groups available in its structure. Porous structure and fiber diameter of nanofabrics contribute to particulate filtration while the rich functional groups on the fiber surface provides toxic chemical filtration property.

First of all, an appropriate solvent, in particular a non-toxic solvent, needs to be selected for preparing the gelatin solution effective for making nanofibers via electrospinning. Many studies have been reported on the fabrication of gelatin fibers by using toxic solvents (TFE, HFIP),\textsuperscript{166,167} which yielded average fiber diameter ranges from 100–600 nm. In this chapter, gelatin nanofabrics were fabricated by employing aqueous acetic acid (AA) as a “green” solvent. In order to achieve efficient molecular dissolution of gelatin, good electrospinnability and, as the result, uniform filter mat with nanoscale fiber diameter, the solvent composition must be adjusted. More importantly,
the uniformity of the nanofibers in the mat along with smaller fiber diameters can result in high surface area for capturing more pollutants, which enables the filter to achieve high filtration efficiency. Therefore, the effect of AA-to-water ratio on the resulting nanofiber diameters and their distribution was studied. The mixture solvent with optimized composition was determined for spinning out uniform gelatin nanofiber mats. The microstructures of gelatin nanofibers prepared in different ratios of the solvent and the fiber morphology of a commercial HEPA filter were compared using scanning electron microscopy (SEM) images (Figure 2.2). It can be seen that by increasing the ratio of AA to water in the solvent from 60 : 40 to 80 : 20, the size of nanofibers was reduced from ca. 470 to ca. 70 nm. Moreover, the nanofiber uniformity was improved significantly: the relative deviation of the nanofibers reduced from 53% to 25% (see fiber diameter distributions and inserted table showing the statistic results in Figure 2.2 and Figure S1.2 for the pore size distribution of nanofibers in Appendix I). These results show that by using the mixed solvent with the AA-to-water ratio of 80 : 20, uniform gelatin filter mats with the nanofiber diameter of 70 nm were successfully fabricated, which is smaller than the reported studies showing the fiber diameter of few hundred nanometers.\textsuperscript{168,172} Reduction in the nanofiber diameter can significantly improve the air filtration capabilities for both particulate and toxic chemicals due to their high active surface areas. In general, nanofibers with high surface-to-volume ratio and surface activity will have high particle removal efficiency via interception, diffusion, and other mechanisms while retaining very low resistance to air flow which results in low pressure drop.\textsuperscript{52,92,173} At the same time, due to the high density of functional groups along the gelatin nanofibers, the nanofiber mats are expected to possess multiple filtering functions: toxic chemical and particulate filtration. The following air filtering performance studies were focused on the gelatin filter nanofibers produced using the optimal solvent.
FIGURE 2.2. SEM images of gelatin nanofabrics prepared in different ratios of AA-to-water; effect of solvent ratio on morphology and fiber diameters of gelatin nanofabrics compared with that of commercial HEPA filter; percentages in the table are relative standard deviation.

2.3.2 Particulate Filtration

As shown in Figure 2.3a, for the gelatin nanofabrics, the removal efficiency is dependent on the PM size as well as the areal density of the nanofabrics. The areal density of the nanofabrics was controlled by controlling the volume of the solution that was electrospun on the substrate. All of the gelatin nanofabric mats possessed a thickness within the range of 8–20 μm. It can be found that with a high areal density (e.g. greater than ca. 3.43 g/m²), the gelatin nanofabrics showed almost similar removal efficiency of above 99.20% for PM with sizes from 0.3 to 10 μm (see Table S1.1 in Appendix I for number concentration of particulate pollutants before and after filtration). In particular, the removal efficiency for the most penetrating particle size (MPPS) of 0.3 μm particles was significantly improved from 77.10 to 99.32% by increasing the areal density of the
nanofabrics from 2.25 to 3.43 g/m². PM particles with size around 0.3 μm (PM_{0.3}) are known as the hardest to capture and a high-performance air filter should give rise to an efficiency above 95% for PM_{0.3}.^{25} The results indicate that the areal density of the nanofabrics is critical for the removal efficiency of small particles, but not large particles. This result can be explained as the difference in the mechanisms for filtering big particles (sieving) and small particles (smaller than the pore size). Specifically, large particles were removed by size effects, while small particles were trapped via the strong interactions between particles and nanofibers. The removal efficiency for PM_{2.5} and PM_{10-2.5} via nanofabrics with different areal density is shown in Figure 2.3b. In particular, the areal density of 3.43 g/m² (thickness = 16 μm) is close to an optimal (minimum) value to achieve the highest removal efficiency of both PM_{2.5} (99.51 ± 0.23%) and PM_{10-2.5} (99.63 ± 0.11%). For the filters with areal densities higher than 3.43 g/m², the removal efficiency of both PM_{2.5} and PM_{10-2.5} was almost constant.

FIGURE 2.3. Particulate removal efficiency of gelatin filter nanofabrics. (a) Particulate removal efficiency of gelatin air filters with different areal density for various PM particle sizes. (b) PM_{2.5}
and PM$_{10-2.5}$ removal efficiency comparison between gelatin air filters with different areal density and commercial HEPA filter.

The gelatin nanofabrics are promising air filtering materials with removing efficiency for PM$_{2.5}$ higher than 95%, the standard suggested for high-efficiency air filters.$^{25}$ More significantly, the gelatin nanofabrics can achieve the high efficiency for PM by a much lower area density (ca. 3.43 g/m$^2$) as compared with the most successful commercial one (HEPA, 164 g/m$^2$). The high removal efficiency for PM is likely contributed by the combination of “nano-size” effects and the surface properties of gelatin nanofibers. The diversity of functional groups on the nanofiber surface can provide strong interactions with the pollutants, which is critical for the removing of PM with sizes much smaller than the pore size of the nanofabrics and of toxic gases as will be discussed later.

Also, the morphology of gelatin nanofibers were studied via SEM and the results are shown by Figure 2.4a–f. In specific, Figure 2.4a and d (also see Figure S1.3a–c, Appendix I) are the SEM images of the nanofabrics before air filtration testing, that is, the morphology of as-spun pure gelatin nanofabrics. Figure 2.4b and d–f (also see Figure S1.3d–f, Appendix I) are the SEM images of the nanofabrics after air filtration testing. The digital photos inserted in the SEM images are the nano-filter before and after filtration. The obvious color change from white to yellowish indicates that the nano-filter has absorbed lots of pollutants. Further, the SEM images confirm this point as one can observe lots of particles have been trapped on the surface of gelatin nanofibers. The notable change in color should also be related to the absorption of some toxic gases, which will be discussed later.
2.3.3 Toxic Gases Filtration

Removing of toxic chemicals via air filters with high efficiency is challenging since they are small molecules with sizes much smaller than that of particles. Conventional ways to remove toxic gases are using absorptive particles with high specific surface area, such as activated carbon. For the gelatin nanofabrics, two kinds of toxic chemicals, formaldehyde (HCHO) and carbon monoxide (CO) which can be detected by the analyzer, were chosen as examples to test the chemical removal capability. Figure 2.5a shows the chemical removal efficiency of formaldehyde for gelatin nanofabrics with different areal densities compared with that of commercial HEPA filter (see Table
S1.2 in Appendix I for concentration of particulate formaldehyde and carbon monoxide before and after filtration).

FIGURE 2.5. Chemical removal efficiency of gelatin filter nanofabrics. (a) Formaldehyde (HCHO) removal efficiency comparison between gelatin air filters with different areal density and that of commercial HEPA filter. (b) Carbon monoxide (CO) removal efficiency comparison between gelatin air filters with different areal density and that of commercial HEPA filter.

It can be seen that the chemical capturing efficiency of formaldehyde increases from 65.0% to 83.0% by increasing the areal density of the filter from 2.25 to 3.80 g/m². Moreover, the removal efficiency of carbon monoxide as shown in Figure 2.5b increased from 62.3% to 76.1% for gelatin nanofabrics with the same change in areal density. In comparison, for the commercial HEPA filter, one of the most successful air filter used today, the chemical removal efficiencies of formaldehyde and carbon monoxide molecules are less than 5 and 3%, respectively. The high chemical removal
efficiency indicates that the combination of nanofabrics with functional polymers is the key to remove toxic chemicals which cannot be removed by size-based mechanisms. Therefore, protein nanofibers provide a promising solution for multi-functional air filtering materials.

2.3.4 Pressure Drop and Figure of Merit (Quality Factor)

In addition to the particulate and chemical removal efficiency, air flow resistance (pressure drop) is another critical parameter describing the performance of an air filter. High pressure drop downstream of an air filter will consume a large amount of energy due to the pumping required to provide a sufficient air flow, which makes the air filter energy consuming. The suggested range for pressure drop (ΔP) set by DOE is less than 1.3 in H2O (approximately 320 Pa).\(^{25}\) The effect of areal density on pressure drop of the gelatin filters was investigated using standard 5 cm/s air face velocity. Figure 2.6a shows that the air flow resistance of the filters increases with the areal density. Quantitative analysis demonstrated that the pressure drop of the gelatin nanofabrics with the lowest areal density (2.25 g/m\(^2\)) was ca. 143 Pa and increased to approximately 201 Pa for the filter with the highest particulate/chemical efficiency (3.43 g/m\(^2\)). These pressure drop values meet the requirement of a high-efficiency filter. The pressure drop along with removal efficiency allow us to determine the optimal areal density of the gelatin nanofabrics for achieving a good balance between high removal efficiency of pollutants (PM and toxic chemicals) and pressure drop, which is critical for practical applications. For the gelatin nanofabrics when the areal density is higher than ca. 3.43 g/m\(^2\), such as 3.80 and 7.67 g/m\(^2\), the removal efficiency is improved by less than 0.5% (see Figure 2.3b), while the pressure drop increases by more than 74% (see Figure 2.6a). Thus, 3.43 g/m\(^2\) should be close to the optimal areal density for the gelatin nanofabrics.
FIGURE 2.6. Pressure drop and overall air filter performance evaluation of gelatin filter material. (a) Dependence of pressure drop (air flow resistance) on the areal density for the gelatin nanofabrics. (b) Quality factor comparison with commercial air filters and transparent PAN air filter, and gelatin air filter.

To comprehensively describe the filtration performance, the pressure drop and removal efficiency is combined into one parameter, the quality factor (QF), also known as figure of merit (FOM), which can be calculated using equation (3).23,174–176 QF is a representative of the ratio between removal efficiency and the air flow pressure drop. This quantitative factor indicates that a good air filter should provide a high removal efficiency and a low pressure drop; hence, a higher QF means a better filtration performance of an air filtering material. Figure 2.6b shows the comparison of figure of merit of the gelatin filter fabrics with commercial air filters and poly(acrylonitrile) (PAN-85) nano-filters as reported recently.18 It can be found that the gelatin nanofabrics with 3.43 g/m² areal density possess the highest quality factor (0.026 Pa⁻¹) among them. The quality factor for gelatin nanofabrics was calculated at 30 minutes of filtration testing and 5 cm/s. It is
noted that the figure of merit of an air filter (including the removal efficiency and pressure drop) is never constant and will change with time of using in practice.

2.3.5 Stages of Filtration Process

For air filtering materials, the analysis of the pollutant absorption process is critical for understanding the long-term filtration performance. For the multi-functional gelatin air filtering material, the pollutant absorption process was tracked quantitatively via calculation of the pollutant weight-percentage gain and pollutant weight absorption rate over time using equation (5) and (6):

\[
W_p = \frac{(W_t - W_f)}{W_f} \quad (5)
\]

\[
W_{p, rate} = \frac{\Delta W_{pollutant}}{\Delta t} \quad (6)
\]

where \( W_p \) is the pollutant weight percentage gain, \( W_t \) is the weight of the air filter after filtration of time \( t \), \( W_f \) is the weight of the pure air filter before filtration test, \( W_{p, rate} \) is the pollutant weight absorption rate, and \( \Delta W_{pollutant} \) is the absolute weight of the pollutants absorbed between each time interval. Results are compared with those of the commercial HEPA filter in Figure 2.7.
At the early stages of filtration (first 30 minutes to 1 hour), particles migrate and merge to form bigger, spherical aggregates. Moreover, the particle weight percentage gain reaches 53% of the nanofabrics' weight and sharply increases after 1 hour of testing to 106% while the HEPA filter only showed an increase from 1% to 1.3% due to its very high areal density (shown in Figure 2.7a). With the increase of filtering time (after 2 hours), more particles were trapped by the nanofibers. The accumulation of particles is also coupled with complex deformation/transformation processes due to possible physicochemical interactions. This process has also been reported in the recent study on transparent PAN air filters. The phenomenon results in a decrease of clear filter area. In this stage, the particle weight percentage gain for gelatin nanofabrics increased moderately to 130%, while the absorption rate of pollutants decreased significantly (shown in Figure 2.7b),
which can prove the hypothesis mentioned above. By further increasing the filtration time, the particle weight percentage gain of the filter reached a plateau region (gelatin nanofabrics and commercial HEPA filter reached to 150% and 2.3% pollutant weight percentage gain, respectively) and the pollutant absorption rate was decreased even more significantly for both filters. However, the pollutant absorption rate of gelatin nanofabrics was higher than that of commercial HEPA filter due the functional and active surface of gelatin nanofabrics which enable it to absorb more particles and chemicals within a shorter time period. The pollutant absorption phenomenon is critical for practical applications as it is related to the long-term performance or the life-time of the air filter material (also see Figure S1.4 and S1.5, Appendix I for SEM images showing the evolution of surface morphology of gelatin nanofabrics during filtration time and thermal stability of gelatin nanofibers before and after filtration).

### 2.3.6 Filtration Mechanism Analysis

To further analyze the performance of the gelatin nanofabrics, the filtration mechanisms were studied based on examining the surface chemistry of pollutants and gelatin nanofabrics before and after filtration. As mentioned above, numerous functional groups exist in gelatin structure. These functional groups can strongly interact with various pollutants in the air and enable the filter to remove the pollutants (toxic chemicals and solid particles) via an interaction-based mechanism besides existing size-based primary mechanisms. Figure 2.8a depicts a simplified gelatin molecule (filter) along with PM, and formaldehyde as examples of solid and gaseous pollutants. It can be seen that the aldehyde group can undergo addition reactions with amine groups in gelatin (provided by amino acids such as lysine) forming Aldimine linkages. This reaction elicits a change in filter color from white to a yellow color. PM particles and other pollutants with different compositions
can interact with the gelatin nanofabrics through hydrogen bonding, charge–charge interactions etc. Figure 2.8b shows an SEM image of gelatin nanofilter that captured numerous pollutants via a combination of interaction-based and size-based mechanisms. This schematic indicates a new interaction-based mechanism of filtration for gelatin nanofabrics besides the primary four size-based mechanisms. To further understand the possible interactions between the gelatin nanofabrics and the pollutants (solid particles and toxic chemicals), Fourier transform infrared spectroscopy (FTIR) and dielectric constant measurement (see Figure S1.6, Appendix I) were used to identify the functional groups from the pure gelatin nanofabrics, polluted air and nanofabrics with trapped pollutants. The FTIR spectra of the cigarette smoke is shown in Figure 2.8c. The specific peaks of functional groups in the cigarette smoke are around 3649, 2360, 1653, 1558, 1506, and 1456 cm\(^{-1}\) which indicate the existence of O–H, C–H (aldehyde), C=O, and C–O (last three peaks) groups, respectively. All of these groups in polluted-air sample may interact with the groups on the surface of gelatin nanofibers. The comparison of the FTIR spectra for the gelatin nanofibers before and after filtration is shown in Figure 2.8d (see Fig. S1.7, Appendix I). It can be found that there is no new peak formed after filtration. However, there is a significant change in the intensity at specific groups and interactions, including hydroxyl, carboxyl, and amine functional groups (see Figure 2.8e). These results can be explained as that the types of interactions between the pollutants in the smoke and gelatin fibers are covered by those inherently existing in gelatin nanofabrics. Therefore, the dramatic increase in the peak intensity of these functional groups after filtration testing should be the result of the interactions between gelatin nanofabrics and the pollutants, such as hydrogen bonding, ionic bonding, and charge–charge interactions, etc.
FIGURE 2.8. (a) Simplified representation of functional-capturing filtration mechanism of gelatin nanofabrics via interactions between gelatin molecules and pollutants. (b) SEM image of gelatin nanofabrics after filtration (c) FTIR characterization of cigarette smoke PM particles demonstrating existing functional groups. (d) FTIR characterization of gelatin filter before and after filtration showing the active functional groups and PM-filter interactions. (e) Functional group peak intensity comparison between gelatin fibers before and after filtration indicating strengthening of corresponding bonds due to PM-filter interactions.

2.4 CONCLUSIONS

In this chapter, the gelatin protein was studied as an example to demonstrate the potential of natural proteins to serve as environmentally friendly and high-performance air-filtering materials. Uniform gelatin nanofiber mats with very small diameters were fabricated by employing a “green”
solvent with optimized composition. It has been found that the gelatin nanofiber mats with a controlled uniformity and small fiber diameters possess extremely high particulate removal efficiencies of more than 99.3% and 99.6% for PM$_{0.3}$ and PM$_{2.5}$, respectively. These results indicate that the gelatin nanofibers with a much lower areal density (e.g. 3.43 g/m$^2$) can efficiently remove a broad range of PM particles similar to one of the most efficient particulate air filters, HEPA with areal density of 164 g/m$^2$. More significantly, the combination of the inherent surface chemistry of gelatin nanofibers (i.e., various functional groups on the fiber surface) and nanofiber technology enables gelatin protein nanofibers to have high interaction capability with toxic chemicals present in the air. Particularly, the gelatin nanofabrics possess excellent efficiency of absorbing toxic chemicals (e.g. ca. 80% for HCHO; 76% for CO), which has never been realized in any air filters with a single material composition. The mechanisms responsible for such simultaneous high capturing capabilities of particulate and toxic chemical were analyzed. It is believed that the interaction-based filtration mechanism besides the existing size-based primary mechanisms result in these functions. The study in this chapter indicates that protein nanofabrics are promising “green” air-filtering materials for next generation air filtration systems.
CHAPTER THREE: SOY-PROTEIN-BASED NANOFABRICS FOR HIGHLY EFFICIENT AND MULTIFUNCTIONAL AIR FILTRATION

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ABSTRACT: Proteins are well-known by their numerous active functional groups along the polypeptide chain. The variety of functional groups of proteins provides a great potential for proteins to interact with airborne pollutants with varying surface properties. However, to our knowledge, a successful demonstration of this potential has not been reported before. In this chapter, soy protein, a type of abundant plant protein, has been employed for the first time to fabricate multifunctional air-filtration materials. To take advantage of the functional groups of soy protein for air filtration, the soy protein was first well denatured to unfold the polypeptide chains and then fabricated into nanofibers with the help of poly(vinyl alcohol). It was found that the resultant nanofabrics showed high filtration efficiency not only for airborne particulates with a broad range of size but also for various toxic gaseous chemicals (e.g., formaldehyde and carbon monoxide), a capability that has not been realized by conventional air-filtering materials. The study is this chapter indicates that protein-based nanofabrics are promising nanomaterials for multifunctional air-filtration applications.
3.1 INTRODUCTION

Air pollution has been a growing concern, and the cleaning of polluted air is becoming increasingly challenging mainly because of the complicated composition of the pollutants, containing particulate matter (PM) of various sizes, chemical vapors, bacteria/viruses, and so on. These complicated air pollutants present discomfort and serious impacts on the health of humans and other living organisms. Most of the particulate pollutants are made of organic compounds, such as carbon-derived species (e.g., CO$_2$ and CO) and sulfur- and nitrogen-based inorganic compounds (e.g., SO$_2^{2-}$, SO$_4^{2-}$, NO$_3^{-}$).$^{12-14}$ Additionally, there are also various toxic gaseous molecules, such as nitrogen dioxide (NO$_2$), methane (CH$_4$), carbon monoxide (CO), formaldehyde (HCHO), and volatile organic compounds (VOCs). These pollutants produced from different sources (such as petrochemical and allied industries) can participate in various photochemical reactions in the atmosphere and create significant environmental hazards.$^{21,22}$ At the same time, these particles and chemicals can form various derived pollutants that can easily penetrate into human lungs and bronchi and cause premature death.$^5$ Therefore, the demand for high-efficiency air-filtering materials that are able to simultaneously capture hazardous particles and chemical gases is increasing dramatically.

Conventionally, synthetic polymers have been employed as air-filtering materials and fabricated into different configurations, such as porous films and nonwoven fibrous mats. Nonwoven mats are typically made of randomly oriented micrometer-size fibers of plastics, such as polyethylene and polypropylene. Usually, these traditional air filter mats can capture particles through only four different size-based mechanisms, including sieving, inertial impaction, interception, and diffusion.$^{50}$ These four mechanisms work together in capturing pollutant particles according to
their sizes. \cite{50,158,159} Clearly, these porous fabrics of conventional plastic micrometer-size fibers are not effective for removing chemical gases because of their inert surfaces and/or limited surface areas. To remove chemical molecules and odors, other filtration materials, such as activated carbon (charcoal), have to be used. As a result, to achieve high efficiency for both particulate and chemical pollutants, the air filters have to combine different layers with different removal functionalities (such as the combination of activated carbon filters with conventional air filters). \cite{157,177,178} This strategy will dramatically increase the air resistance or pressure drop and, as a result, the energy consumption of the air filter. Therefore, developing a single material with multifunctional filtration properties is significant for the development of cost-effective and high-efficiency air filters.

Soy protein (SP), one of the most abundant and low-cost plant proteins, has been widely studied as a type of biomaterial with different applications, including antibacterial agents, \cite{179} active food packaging, \cite{180} adhesives, \cite{181,182} tissue engineering, \cite{183} and drug delivery. \cite{184} These significant applications of SP indicate that SP is a high-performance biomaterial with multi-functionality. In particular, its ionizable groups, such as glutamic acid, lysine, and histidine, \cite{185} have been found to be critical for antibacterial properties. \cite{186,187} It was reported that the ionizable groups in soy protein can create active sites to capture bacteria. \cite{179} Because charged fibers have been reported to be very effective for capturing various types of pollutants, \cite{188,189} the presence of these ionizable groups in soy protein indicates a great potential to capture charged pollutants. In fact, it is known that, in addition to these ionizable groups, soy protein contains many other functional groups, including polar, nonpolar, hydrophobic, and hydrophilic ones. These functional groups, such as hydroxyl (−OH), carboxyl (−COOH), amine (−NH₂ and −NH₃⁺), and methyl (CH₃) groups, make soy protein a very attractive material with the ability to interact with various particles or chemicals. To
demonstrate this great potential, in this chapter, soy protein is first denatured and then fabricated into nanofibers with the help of poly(vinyl alcohol). The denaturation combined with the nanofiber morphology can greatly increase the density of active sites available for interacting with pollutants. The results revealed that SP-based nanofabrics can provide high removal efficiencies for both particulate and chemical pollutants, which, to the best of our knowledge, has never been realized in a single material before.

3.2 EXPERIMENTAL

3.2.1 Raw Materials and Solution Preparation

Soy protein isolate powder with >90% protein content was supplied from ADM Foods & Wellness, Decatur, IL. Granules of poly(vinyl alcohol) (PVA; Mn = 75000 g/mol) were obtained from Sigma-Aldrich, St. Louis, MO. Acetic acid (99.9% purity) was purchased from J.T. Baker (Center Valley, PA). Two main procedures were explored in this chapter to produce the nanocomposite fibrous mats, a powder-based procedure and a solution-based procedure. It was found experimentally that samples prepared by the solution-based procedure consistently outperformed the samples prepared by the powder-based procedure. The sample preparation and particulate and chemical filtration data (see Figures S2.1 and S2.2) for the powder-based procedure are provided in the Appendix II. Because of the issues that occurred during implementation of the powder-based procedure, the solution-based procedure was chosen for the studies presented in this chapter. For the solution-based method, soy protein isolate (SPI) was thermally denatured in mixed solvent (acetic acid/deionized water = 80:20, v/v) at a concentration of 8.5 wt % at 85 °C for 4 h using magnetic stirring (400 rpm). Poly(vinyl alcohol) was dissolved separately in the same solvent at a concentration of 8.5 wt % at 60 °C for 2 h using magnetic stirring (400 rpm). Then, the denatured
SPI was loaded as a solution into the PVA solution at different ratios and mixed with the PVA solution for 24 h using a spin mixer.

### 3.2.2 Preparation of Soy-Protein-Based Filter Nanofabrics

The soy-protein-based nanofibers were fabricated by the electrospinning technique. The SPI/PVA nanocomposite solution was loaded in a syringe (Monojet Kendall) with a 21-gauge blunt-tip needle. A voltage of 16–21 kV was applied for electrospinning and was controlled by a high-voltage power source (ES50P-5W, Gamma High Voltage Research). A monoinject syringe pump (KD Scientific, KDS-100) was used to pump the SPI/PVA solution. Commercial aluminum mesh with a wire diameter of 0.011 in. and a mesh pore size of 1 mm × 1 mm was grounded to collect the fibers. The distance between the needle and the sample collector was fixed at 10 cm, and an average flow rate of 0.6 mL/h was utilized. Moreover, the needle position (horizontal and vertical) was adjusted continuously during electrospinning to achieve a uniform fiber mat with a controlled nanofiber diameter and different areal densities.

### 3.2.3 Pollution Generation and Air Filtration Testing

Two different sources of pollution were utilized to prepare polluted air samples: cigarette smoke and the air product of burning plant materials. It has been established that cigarette smoke consists of PM with sizes ranging from 0.01 to 10 μm and approximately 7000 different chemicals, of which hundreds are toxic such as formaldehyde (HCHO) and carbon monoxide (CO). Samples produced from burning plant materials also included a broad range of PM and high concentrations of HCHO and CO. The polluted air samples were diluted in a plastic gasbag to a
hazardous and measurable level for the analyzer because of the very high initial pollutant concentration. A particle counter (CEM, DT-9881) was used to measure the concentrations of PM (different particle sizes from 0.3 to 10 μm) and toxic chemicals (HCHO and CO) in the polluted air samples. Also, the air-flow resistance (the pressure difference between the two sides of the air filter) was controlled and measured by a monometer (UEi, EM201-B) with a standard air-flow velocity of 5 cm/s.\textsuperscript{25} A circular filter sample with a diameter of 37 mm was placed in a homemade sample holder to perform air-filtration testing for all of the measurements. The air downstream of the filter was collected in a clean vacuum gasbag. Similar measurements were conducted for the collected filtered air downstream of the filter. The testing procedure was performed on four filters fabricated independently (with similar areal densities) from the same solution for each type of samples to replicate the results. The removal efficiency $\eta$ was determined from equation (1).

### 3.2.4 Characterization

Scanning electron microscopy (SEM, FEI SEM Quanta 200F) was utilized to investigate the change in SPI particle size after the denaturation process, as well as morphologies of the nanofabrics before and after air filtration. All samples were sputter-coated with a gold nanolayer (10 nm in thickness) using a Technics Hummer V sputter coater. To further study the denaturation of SPI, transmission electron microscopy (TEM, FEI Tecnai G2 20 Twin) was used to investigate the particle size. To study the interface interactions between pollutants and nanofabrics, Fourier transform infrared (FTIR, Nicolet Thermo Scientific) spectroscopy was employed. To distinguish the interactions between nanofibers and pollutants from the interactions within the fabric or polluted-air samples themselves, the FTIR spectra of polluted air and clean protein nanofabrics before and after filtration were compared. Each of the measurements was repeated three times.
3.3 RESULTS AND DISCUSSION

3.3.1 Denaturation of Soy Protein

To explore the potential of soy protein isolate (SPI) for air-filtration applications, the protein was first denatured to unfold the built-in protein structures. As illustrated in Figure 3.1a, pristine soy protein takes the form of micron-size powders with a diameter of about 50 μm (see Figure 3.1b). It is well-known that natural proteins usually have four different levels of structure,\textsuperscript{191,192} including primary, secondary, tertiary, and quaternary structures. For simplicity, the raw material, pristine SPI, can be viewed as a large particle held by numerous intermolecular interactions contributed by 18 types of amino acids in the protein chains.\textsuperscript{191} Therefore, the denaturation process can destroy the built-in high-level structures (secondary, tertiary, and quaternary) of the large SPI particles and finally unfold the soy protein chains, as illustrated in Figure 3.1c. To study the effect of the denaturation process on the SPI morphology, scanning electron microscopy (SEM) and transmission electron microcopy (TEM) were utilized. SEM and TEM images of the denatured SPI particles are shown in panels d and e, respectively, of Figure 3.1. One can see that denaturation can result in a significant reduction in particle size from about 50 μm to less than 30 nm. The huge reduction in the particle size indicates that more functional groups along the SPI chain will be exposed to the surface, which is critical for air-filtration applications. At the same time, a well-denatured SPI is also important for the electrospinning process, as well as for the quality control of the nanofibers, because it is one of the prerequisites for achieving good homogeneity for both the SP/PVA solution and the final nanofibers.
3.3.2 Morphology of SP/PVA Nanofabrics

As described in the Experimental section above, cigarette smoke was used as a sample of polluted air. Cigarette smoke includes a very complicated combination of various particles, toxic chemical molecules, and even heavy-metal ions. This mixture of various particles and hazardous chemical molecules makes cigarette smoke a good sample for the evaluation of the filtration performance of the SP/PVA nanofabrics. SEM was utilized to investigate the morphological characteristics of nanofibrous filter mats with different SPI concentrations and similar areal densities (4.50 g/m²).
Figure 3.2a,c,e shows SEM images of SPI/PVA nanofabrics with different SPI/PVA ratios before air-filtration testing. From the SEM images, it can be seen that all of the SPI/PVA ratios gave rise to similar fiber diameters in the range of 100–200 nm (see Figure 3.2g and Figure S2.4 in Appendix II for the size distribution). It is noted that the pores in the nanofabrics were irregular, making it difficult to determine the pore geometry. As a result, the pore size was estimated by ImageJ, and the size distributions of these samples were found to be similar, ca. 4 μm, as shown in Figure 3.2h (also see Figure S2.3, Appendix II). To characterize the distribution of the denatured SPI in the SPI/PVA nanofibers, high-magnification images of the nanofibers were used to characterize the morphological structures of single SPI/PVA nanofibers (see the TEM images in Figure S2.4, Appendix II). Based on the images, one can see that there were no SPI nanoparticles inside the nanofibers or on the nanofiber surface, indicating a good miscibility between denatured SPI and PVA, which is consistent with other studies.\cite{193,194} Nevertheless, it is still believed that there are SPI molecules on the nanofiber surface for the following reasons: (1) The air-filtration performance of the SPI/PVA nanofabrics, which will be introduced later, is much better than that of pure PVA nanofabrics, indicating that the surface of PVA nanofiber is very different from that of the PVA/SPI nanofibers. (2) The TEM images (see Figure S2.5, Appendix II) show that there is no core–shell structure of the SPI/PVA nanofiber, indicating SPI is homogeneously distributed in the nanofibers and that many SPI molecules exist on the surface because a high loading of SPI was used for the nanofibers.
FIGURE 3.2. (a–f) SEM images of SPI/PVA air filter nanofabrics (a,c,e) before and (b,d,f) after filtration with nanofabrics with different SPI/PVA ratios: (a,b) 0:1, (c,d) 1:1, and (e,f) 1.5:1. Insets in panels c and d: Digital photographs of the air filter with an SPI/PVA ratio of 1:1 (c) before and (d) after the air-filtration testing. Scale bar = 1 μm. (g) Fiber diameter and (h) pore size distributions of SPI/PVA the air filter with SPI/PVA = 1:1.

Figure 3.2b,d,f presents SEM images of the nanofabrics with different SPI/PVA ratios after air-filtration testing. The digital photographs in Figure 3.2c,d (insets) display a dramatic color change from white (clean filter before air filtration) to yellow/orange (used filter after air filtration), indicating that the SPI/PVA nanofabrics captured many pollutants. Although the morphologies of the as-spun nanofibers with different SPI concentrations were mostly similar, the SEM images after filtration showed that the soy-protein-based filter mats can capture more particles after exposure to the same polluted air for the same time (see Figure 3.2b,d). More SEM images of SPI/PVA nanofabrics with other ratios can be found in Figure S2.6 (Appendix II). It was found
that the sample with an SPI/PVA ratio of about 1:1 captured more particles than the rest of the samples, which indicates an optimal loading of SPI for good air-filtration performance. The existence of an optimal SPI loading is mainly due to the fact that the air-filtration performance is determined by two factors related to SPI loading at the same time, namely, the mechanical strength and surface activity of the nanofabrics, which will be further discussed in the next section.

3.3.3 Air Filtration Performance

To evaluate the filtering capabilities of the protein-based nanofiber mats, we performed both efficiency (η, %) testing and pressure-drop (ΔP, Pa) testing. For a standard high-efficiency filter, the PM$_{2.5}$ efficiency is suggested to be 95–100%, and for a high-efficiency particulate air (HEPA) filter, the requirements state that it must fulfill a removal efficiency of 99.97% for the most penetrating particle size (MPPS) of 0.3 μm with a maximum pressure drop of 1.3 in H$_2$O gauge (~325 Pa) at an air face velocity of 5 cm/s, as suggested by the US Department of Energy (DOE).\textsuperscript{25}

3.3.3.1 Removal of Particulate Pollutants

In this chapter, we first focused on the effect of the SPI concentration on the morphology of the nanofabrics, as shown in Figure 3.2. The PM$_{2.5}$ and PM$_{10-2.5}$ filtration efficiencies of these samples were then tested. To compare the performance, all of these samples were prepared with similar areal densities (ca. 4.5 g/m$^2$). As shown in Figure 3.3, the removal efficiencies for small particles (PM$_{2.5}$, that is, particles with sizes less than 2.5 μm) and large particles (PM$_{10-2.5}$) are compared for SPI/PVA nanofabrics with different SPI/PVA ratios. Based on Figure 3.3, it can be concluded that the removal efficiency of PM$_{10-2.5}$ (particles with sizes between 2.5 and 10 μm) stays in the
same range of about 99.90–99.99%, regardless of the SPI loading. This result indicates that large particles are mostly captured through sized-based physical mechanisms of filtration. However, for PM$_{2.5}$, the removal efficiency as displayed in Figure 3.3 is dependent on the SPI/PVA ratio and lies in the range of 99.40–99.80%. In particular, the neat PVA nanofabric mats show a PM$_{2.5}$ removal efficiency of 99.45%, whereas the SPI/PVA nanofabrics with a 1:1 ratio leads to a higher efficiency of 99.80% for PM$_{2.5}$. It is noted that an improvement of 0.4% is significant because the efficiency is approaching the limit of 100%. The above results reveal that the contribution of SPI is critical for improving the removal efficiency for small particles with sizes smaller than the pore size (ca. 4 μm). This result also indicates that the small particles were removed by the interaction-based mechanism with the critical contribution from the multiple functional groups in the SPI structure, instead of the size-based mechanisms. This conjecture will be further confirmed by the filtration performance of toxic chemicals, as discussed later.

![Bar chart showing particulate removal efficiencies of neat PVA and SPI/PVA air-filter nanofabrics with different SPI contents.](image)

**FIGURE 3.3.** PM$_{2.5}$ and PM$_{10-2.5}$ particulate removal efficiencies of neat PVA and SPI/PVA air-filter nanofabrics with different SPI contents.
From Figure 3.3, it can also be seen that the sample with the PVA/SPI ratio of about 1:1 gives rise to the best air-filtration performance in terms of removing PM$_{2.5}$. This result can be explained as follows: The filtration performance of the composite nanofabric is dependent on not only the PVA/SPI ratio but also the mechanical strength of the nanofabrics. It was found that, when the SPI/PVA was higher than 1:1, the PVA/SPI nanofibers became brittle, leading to the formation of micro-cracks or large pores in the nanofabrics after electrospinning or during filtration testing (see Figure S2.7, Appendix II), which is consistent with other studies.$^{193,195,196}$ As a result, there should be an optimal loading for SPI to achieve a good balance between mechanical strength and surface activity. In brief, the structure and mechanical weakness of PVA/SPI nanofibers with high SPI/PVA ratios (higher than 1:1) are the main reasons for the efficiency drop. Therefore, in the following studies, all samples had a PVA/SPI ratio of 1:1.

In addition to the SPI/PVA ratio, another critical parameter affecting the air-filtration performance is the areal density of the nanofabrics. In Figure 3.4a, the removal efficiencies for pollutant particles with different sizes are compared among nanofabric samples with different areal densities. From these results, it can be seen that, for particles with sizes larger than ca. 1 μm, the removal efficiency did not change significantly with increasing areal density. However, for particles with sizes less than 1 μm, the removal efficiency was found to be highly dependent on the areal density until this density reached ca. 4.50 g/m$^2$, at which point the removal efficiency reached its maximum value. For the very small particles of PM$_{0.3}$, an improvement in removal efficiency from 86.40% to 98.70% was achieved when the areal density was increased from 1.55 to 4.50 g/m$^2$. However, no significant increase in removal efficiency for PM$_{0.3}$ could be found when the areal density was greater than 4.50 g/m$^2$. These results show that increasing the areal
density of nanofabrics can markedly improve the removal efficiency of small particles but not larger particles. This phenomenon can be explained as follows: First, removal efficiency is probably determined by an interaction-based mechanism for particles that have sizes smaller than the pore size of the nanofabrics. Second, a higher nanofabric areal density will increase the contact possibilities between the small particles and the nanofabrics, as well as the chance of capturing more small particles. Figure 3.4b further displays how the areal density affects the removal efficiencies for PM$_{2.5}$ and PM$_{10-2.5}$. From this figure, it can be seen that the areal density does not affect the removal efficiency for PM$_{10-2.5}$ much, but it affects that for PM$_{2.5}$ significantly, which is similar to the situation for PM$_{0.3}$ as mentioned previously. It can also be seen that, above an areal density of 4.50 g/m$^2$, there was no significant improvement in the removal efficiency of PM$_{2.5}$. However, below 4.50 g/m$^2$, the PM$_{2.5}$ removal efficiency increased with increasing areal density. Therefore, it can be concluded that an appropriate areal density of protein nanofabrics is important for achieving a high removal efficiency for small particles. The high removal efficiency for small particles is most likely due to the “nanosize” effects and “active” surface properties of the protein-based nanofibers, which can help to trap very small particles with sizes below the pore size of the nanofabrics.
FIGURE 3.4. Particulate removal efficiencies of SPI/PVA air filters with different areal densities. (a) Particulate removal efficiencies for various PM particle sizes. (b) Effect of the areal density on the PM$_{2.5}$ and PM$_{10-2.5}$ removal efficiencies.

3.3.3.2 Removal of Toxic Chemicals

In addition to a high efficiency for removing PM with different sizes, the protein-based nanofabrics also exhibited excellent removal efficiencies for toxic chemicals. In this chapter, we chose formaldehyde (HCHO) and carbon monoxide (CO) molecules to test the chemical removal performance of the protein-based nanofabrics. HCHO and CO are cancer-causing and poisonous gases that exist in cigarette smoke. Figure 3.5a summarizes the chemical removal efficiencies of HCHO and CO for nanofabric samples with different SPI/PVA ratios. For the HCHO removal efficiency, this figure demonstrates that the overall range of HCHO removal efficiency was between 30.0% and 62.50%. In particular, the neat PVA nanofabric showed a much lower HCHO removal efficiency (ca. 31.23%) than SPI/PVA nanofabrics (ca. 62.50% for the sample with an SPI/PVA ratio of 1:1), indicating that the SPI plays a critical role in removing chemical gases. For
the CO removal efficiency, all of the nanofabrics, including pure PVA, showed much better performance than for HCHO. The removal efficiencies of the SPI/PVA samples ranged from 76.90% to 90.90%, and that of the pure PVA sample was only 55.67%, much lower than those of the SPI/PVA samples. Similarly to the particulate removal efficiency, it was found that there was an optimal PVA/SPI ratio that gave rise to the best air-filtration performance in terms of removing toxic chemicals. As a comparison, the removal efficiencies for HCHO and CO by commercial HEPA filters, which have no functional groups along their fibers, were also tested. As shown in Figure 3.5a, the removal efficiencies for HCHO and CO were less than 5% and 3%, respectively, even though these filters have a much higher areal density of 164 g/m². Because toxic gases are molecules with sizes much smaller than the particles in polluted air, it is believed that the removal of toxic chemicals is governed by an interaction-based filtration mechanism that is contributed by the soy protein structure, as discussed later.
FIGURE 3.5. Formaldehyde and carbon monoxide removal efficiencies for neat PVA and SPI/PVA nanofabrics: (a) effects of SPI concentration and (b) effects of areal density for the SPI/PVA sample with a ratio of 1:1.

The effects of areal density on the chemical removal efficiencies for HCHO and CO are shown in Figure 3.5b. The results show that the removal efficiencies for these toxic gases did not change significantly with increasing areal density of the nanofabrics. The removal efficiency of HCHO fluctuated between 42.50\% and 62.50\%, and that of CO fluctuated between 78.90\% and 85.70\%. These results might suggest that the removal of toxic gases is a very slow process and that a change in areal density does not lead to a significant difference in the time required to absorb toxic gases. From the above results, one can conclude that the combination of nanofiber mats with functional SPI on the surface provides a promising solution for multifunctional air-filtering materials.
### 3.3.3.3 Pressure Drop and Quality Factor

In addition to particulate and chemical removal efficiencies, pressure drop or air-flow resistance of an air filter is another parameter related to filtration performance. As suggested by the U.S. Department of Energy, the pressure drop should be less than ca. 325 Pa at an air face velocity of 5 cm/s. Figure 3.6a shows a quantitative study on how the air-flow resistance changed with the areal density of the sample with an SPI/PVA ratio of 1:1. It can be seen that the pressure drop generally increased nonlinearly with the areal density, which might be related to complicated changes in the porous structure of the nanofabrics when increasing numbers of layers of nanofibers are added. Taking into account the particulate removal efficiency (see Figure 3.3) and the pressure drop (see Figure 3.6a), the optimized areal density was determined experimentally to be about 4.50 g/m². It is noted that the optimal areal density should also be related to the size of the nanofibers as well as the porous structures of the nanofabrics, which is beyond the scope of this study. To correlate the pressure drop and removal efficiency for evaluating the overall performance of the SPI/PVA nanofabrics, a quality factor (QF), that is, a figure of merit (FOM), was calculated for the filtering materials studied in this work. The QF is related to the ratio between the particulate removal efficiency ($\eta_p$) of the air filter and the pressure drop ($\Delta P$) due to air flow across the filter and represents a comprehensive evaluation of the air-filtering performance. For a good air filter, the value of QF should be high, which means that the air filter can achieve a high removal efficiency with a low pressure drop or air resistance. A QF comparison among different types of air-filtering materials and the SPI/PVA nanofabrics is shown in Figure 3.6b. Data for two types of commercial air filters along with the reported PAN-85 air filter are included in this figure for comparison. This comparison demonstrates that the optimized PVA/SPI nanofabric exhibits the
highest QF (ca. 0.027 Pa⁻¹) among these different types of air-filtering materials, which indicates that the protein-based nanofabric provides the best filtration performance.

![Figure 3.6](image)

**FIGURE 3.6.** Pressure drops and quality factors of SPI/PVA nanofabric filters. (a) Pressure drops (air-flow resistance) for the optimized SPI/PVA air filters with different areal densities and (b) comparison of the quality factors among a regular commercial air filter, a commercial HEPA filter, a transparent PAN air filter, and the SPI/PVA nanofabric filter with a ratio of 1:1 and an areal density of 4.50 g/m².

The time-dependent behavior of the filtration performance of the SPI/PVA nanofabrics was also studied in this chapter. Time-dependent behavior is related to the long-term performance of an air-filter material. For the protein-based nanofabrics, the time-dependent air- filtration performance was studied by investigating how the removal efficiency and weight gain of pollutants depend on the time of use. For simplicity, only the optimized SPI/PVA nanofabric was employed in a
comparison with other filters. The filters were exposed to highly polluted air from cigarette smoke for about 180 min. After each time interval of 45 min, the particulate and chemical removing efficiencies and the weight gain of pollutants were recorded. As shown in Figure 3.7a, the particulate removal efficiencies for both the SPI/PVA nanofabric and the commercial HEPA filter decreased with testing time. It can be seen that the PM$_{2.5}$ removal efficiency for the SPI/PVA nanofabric decreased slightly faster than that for the commercial HEPA filter. This is because the commercial HEPA filter had a much higher areal density (ca. 164 g/m$^2$) than our SPI/PVA nanofabric (4.5 g/m$^2$). Figure 3.7b displays the toxic chemical removal efficiencies as a function of testing time for the same two filters. For the protein nanofabric sample, the HCHO removal efficiency dropped steadily from 63.28% to 34.78% after 45 min of testing time, whereas the CO removal efficiency dropped from a high value of 85.78% to 62.53% after 45 min and further to 33.33% after 90 min of testing. In comparison, for the commercial HEPA filter, the chemical removal efficiencies for both HCHO and CO were below 5% and decreased to less than 3% after about 180 min of testing. This decreasing behavior of the removal efficiency is mainly due to the fact that fewer active sites on the nanofiber surface are available for pollutant absorption as the filtration process continues.
FIGURE 3.7. Study of the time-dependent behavior of the filtration performance of the SPI/PVA nanofabrics: (a) PM$_{2.5}$ filtration efficiency, (b) toxic chemical filtration efficiency, and (c) relative weight gains from the pollutants.

To further demonstrate the advantages of the SPI/PVA nanofabric in removing pollutants, the weight gains of captured pollutants were recorded after each time interval. It was found that the total weight of captured pollutants on the SPI/PVA nanofabric increased from 3.5 to 7.3 mg after 180 min of testing. In contrast, the commercial HEPA filter showed only a slight weight increase from 2 to 3.8 mg after the same testing time. To better demonstrate this advantage of the protein-
based nanofabrics, we use the ratio $W_p/W_f$, where $W_p$ is the weight gain of captured pollutants and $W_f$ is the weight of the filter before testing, to describe the ability to capture pollutants. It can be seen in Figure 3.7c that the ratio $W_p/W_f$ for the PVA/SPI nanofabric increased from 0.72 to 1.5 as the testing time was increased from 5 to 180 min. In contrast, the $W_p/W_f$ ratio for the HEPA filter increased from only 0.01 to 0.02 for the same time interval. A high $W_p/W_f$ ratio indicates a strong ability to capture pollutants. The value of 1.5 for $W_p/W_f$ reveals that the SPI/PVA nanofiber can capture an amount of pollutants with a weight even much higher than the weight of the filter material itself. This behavior is similar to that of a spider web, which has very low weight but can capture huge particles. The above results indicate that the protein-based nanofabrics can simultaneously improve both the removal efficiencies for particulates and toxic gases and the long-term performance as a result of an enhanced capturing mechanism for both particles and toxic gases.

### 3.3.3.4 Filtration Mechanism

To further understand the unique performance of the SPI/PVA nanofabrics, we propose an interaction-based capture mechanism based on an examination of the chemical characteristics of both the cigarette-smoke pollutants and the nanofabrics before and after filtration testing. Conventionally, the removal efficiency for particles is mainly dependent on the morphology of the filter mats because of the four primary sized-based filtration mechanisms. As described in chapter one, compared with micrometer-sized fibers, nanofibers have larger surface areas and higher surface energies, which can dramatically improve their interactions with PM particles and enhance their efficiencies. Moreover, as mentioned earlier, SPI contains numerous functional groups that can interact with different types of particles and toxic chemicals in polluted air. The strong
interactions between PVA/SPI nanofabrics and pollutants (see Figure 3.8a) enhance the capturing capabilities for both toxic chemicals and solid particles. A simplified schematic illustration of the possible interactions among PVA, SPI molecules, PM particles, and formaldehyde (as examples of different pollutants in the air) is shown in Figure 3.8a. Figure 3.8b presents an SEM image showing SPI/PVA nanofabrics strongly interacting with pollutants after a filtration test. It can be seen that the aldehyde groups in formaldehyde can interact with both carboxylic and amine groups of the soy protein. This interaction can result in the formation of Aldimine bonds, which is the main reason for the color change of the filter to yellowish after the filtration test (see the inset photographs in Figure 3.2). Moreover, solid particles and other toxic chemicals with different compositions can undergo various types of interactions including charge–charge, polar–polar, and hydrogen-bonding interactions. This schematic demonstrates an enhanced mechanism based on strong fiber–pollutant interactions.

To further characterize the interactions between pollutants and protein-based nanofibers, Fourier transform infrared (FTIR) spectroscopy was employed to investigate the functional groups existing in the polluted air, clean SPI/PVA nanofabrics, and nanofibers with captured pollutants. The FTIR spectra of the cigarette smoke is shown in Figure 3.8c. The main peaks are at about 3649, 2360, 1653, 1558, 1506, and 1456 cm\(^{-1}\), which indicate the presence of O—H, C—H:H—C═O (formaldehyde), C═O, and C—O (last three peaks) groups, respectively. These groups in polluted air can strongly interact with the functional groups on the surface of the nanofibers. By comparison of the FTIR spectra of neat PVA and SPI/PVA nanofibers (see Figure 3.8d), one can easily identify the SPI in the nanofibers by amide-group signals. Although no new peaks are generated between the SPI/PVA nanofabrics and the pollutants, significant changes in the intensities of the peaks for
specific groups/interactions (e.g., O—H, COOH, N—H, and C—N functional groups) were observed, as summarized in Figure 3.8e. The reason no new peaks were generated after the filtration testing is possibly that the interactions between the pollutants and the SPI/PVA nanofabric are covered by those interactions existing in the protein. As a result, one can observe only increases in peak intensity, instead of new peaks, after filtration.

FIGURE 3.8. Study of the filtration mechanism of the PVA/SPI nanofabrics. (a) Simplified representation of the interaction-based filtration mechanism for soy-protein-based nanofabrics. (b) SEM image of SPI/PVA nanofabric covered by pollutants. (c) FTIR characterization of cigarette-smoke PM particles demonstrating the functional groups. (d) FTIR characterization of neat PVA and SPI/PVA nanofabrics before and after filtration. (e) Change in the peak intensities of functional groups/interactions responsible for the pollutant–nanofiber interactions.
3.4 CONCLUSIONS

In summary, this chapter demonstrates a high-performance multifunctional air filtration nanofabric material produced from protein/polymer composites. The combination of abundant plant protein with porous nanofabrics provides a promising solution for the production of “green” and highly efficient nanomaterials for air-filtration applications. The protein-based nanofabrics show high removal efficiencies for both types of pollutants, namely, particles with a broad size range and toxic gases with various characteristics, which has never been reported for a conventional air-filtering material. The soy protein was employed as an example to develop sustainable and environmentally friendly nanomaterials for air-filtering applications. The study in this chapter indicates that the amino acids of proteins can significantly enhance the interactions between nanofabrics and pollutants, which is especially critical to the capture of particles with sizes much smaller than the pore size and of gaseous molecules. Moreover, the protein-based nanofabrics are able to improve the removal efficiency of air pollutants while decreasing the air-flow resistance, both of which are the most crucial factors for practical applications. In short, the study in this chapter indicates that protein-based nanofabrics represent a promising green nanomaterial with great potential to deal with complicated pollutants in the air through an enhanced-interaction mechanism.
CHAPTER FOUR: A DISPOSABLE MULTIFUNCTIONAL AIR FILTER: PAPER TOWEL/PROTEIN NANOFIBERS WITH GRADIENT POROUS STRUCTURES FOR CAPTURING POLLUTANTS OF BROAD SPECIES AND SIZES

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ABSTRACT: Highly polluted air is usually concentrated with particles of broad sizes and species of gaseous toxic chemicals. Filtration of these pollutants simply relying on size effects is not sufficient; instead, strong interactions between filtering materials and pollutants are in critical need. Moreover, to avoid further pollution to environment from disposing the massive amount of used air filters demands development of “green” air filtering materials. In this chapter, we report a high-performance hybrid cellulose/protein air-filter with nanofiber structures. It is interestingly discovered that the textured cellulose paper towel not only can act as a flexible mechanical support, but also a type of air flow regulator which can improve the pollutant-nanofilter interactions. Therefore, the high-performance natural protein-based nanofabrics are promoted both mechanically and functionally by a textured cellulose paper towel. Study results indicate that this hybrid filtering material possesses excellent removal efficiency for particulate matter and multiple
species of toxic chemicals effectively. It indicates that protein/cellulose hybrid system can be used in high-performance air-filters, and are disposable due to the abundance and environmental friendliness of the material origins.
4.1 INTRODUCTION

Highly polluted air become a major environmental concern in the areas with a huge amount of pollutants produced from various human activities. The polluted air usually contains numerous combinations of pollutants such as particle matter (PM) of various sizes, chemical mixtures, and various biological hazards, etc. According to the size of particulate pollutants, the particulate pollutants can be classified into different levels, such as PM\(_{2.5}\) with size less than 2.5 \(\mu\)m and PM\(_{10-2.5}\) with size in the range of 2.5 – 10 \(\mu\)m. PM\(_{2.5}\) is well-known as the major pollutants, which seriously influences the air quality, participates in climate change and decreases the air visibility and so on. In addition to the complicated particulate pollutants, polluted air usually includes numerous types of toxic gaseous molecules with sizes at molecular level, such as sulfur oxides (SO\(_x\)), nitrogen oxides (NO\(_x\)), carbon oxides (CO and CO\(_2\)), formaldehyde (HCHO), methane (CH\(_4\)), and mixtures of other volatile organic compounds (VOCs). These chemical and particulate pollutants can generate new pollutant species by some complicated chemical reactions, such as photochemical reactions induced by the sunlight. These uncontrolled reactions lead to many unpredicted toxic pollutants, and thus make the compositions of polluted air even more complicated and posing excessive threats to public health. To incorporate certain fillers into the air filters that can only work for the known pollutants is unable to solve the issues relevant to the pollution complexity with the unexpected hazardousness in the air. Therefore, development of high-performance air-filters which can simultaneously remove particulate (especially small particulate pollutants, such as PM\(_{0.3}\) and more species chemical pollutants with high efficiency is in critical need.
However, a high efficient filtration of small particulate pollutants (such as PM$_{0.3}$) and chemical gases has been very challenging for the limitations on two different levels. For the first level, that is, the materials level, the materials employed for current air-filters show insufficient interactions with the pollutants. For example, conventional air filters are usually made of micron-size fibers of synthetic plastics such as polyethylene and polypropylene. These air filters are ineffective in removing toxic gaseous chemicals from the air due to the lack of active functional groups on the surface of the fibers. They are usually designed to remove particulate pollutants based on the four primary physical filtration mechanisms, including sieving, interception, impaction, and diffusion.\textsuperscript{50} It is noted that, even for particulate pollutants, these filtration mechanisms are not effective for removing the small particles with size much smaller than the pore size, which represents another limitation of the conventional air-filter materials. For the second (device) level, the functions enabled by strong pollutant-filter interactions for removing small particulate pollutants and chemical gases will deteriorate fast during the filtration, which is particularly true for the case of highly polluted air. Therefore, an effective solution to the above issues has to simultaneously address the limitations at these two levels. In previous chapters,\textsuperscript{27,28} we have developed a natural protein-based nanofabrics with multifunctional air filtration capabilities due to a high affinity/interactions toward various pollutants. Benefited from the strong interactions between pollutants and the protein nanofabrics, the protein-based nanofabrics showed high removal efficiencies for both small solid particles and various toxic gaseous chemicals while maintaining a very low resistance to air. These previous studies provide a good prototype for solving the issues on material level. However, the protein nanofabric is mechanically weak and more efforts should be on addressing the issues on the device level.
In this chapter, we propose a new strategy based on paper towel to address the above issues simultaneously. Paper towel is a porous film made of cellulose, the most abundant polymer in nature with low price and high biodegradability. The porous fibrous structure of cellulose leads to low air-resistance, high mechanical strength as well as good flexibility. In fact, cellulose has been studied extensively as wastewater treatment filtration membranes, films, hydrogels and aerogels, and energy harvesting. In addition, cellulose is also rich in polar groups which can be employed as a functional reinforcement for the protein nanofabrics possessing numerous amino acids in their chemical structure. The studies in previous chapters showed that protein nanofabric can strongly interact with various pollutants and give rise to high removing efficiencies for both particulate and toxic chemical pollutions. We believe that a combination of porous paper towel with protein nanofabric can potentially further improve the performance and mechanical properties of the protein. Since both the protein and paper towel are abundant natural materials, the resultant nano-filter can be used as a high performance, extremely low-cost and disposable material for air-filtration applications.

4.2 EXPERIMENTAL

4.2.1 Raw Materials and Gelatin Solution Preparation

Three types of cellulose-based paper towels (Scott® PT-T (textured), Scott® PT-P (plain), and Bounty® PT-B) with different surface morphology/texture were investigated first to determine the air-filtration performance of PT. Gelatin powder (type A, from porcine skin) was purchased from Sigma Aldrich. Glacial acetic acid (AcOH, purity = 99.9%) was purchased from J.T.Baker® (PA, USA). The gelatin solution was prepared in a mixture of AcOH : DI water with a 80:20 volume
ratio to achieve a homogenous yellow solution to be used to deposit the active nanofiber layer by electrospinning.\textsuperscript{28}

### 4.2.2 Preparation of Protein Nanofiber-Coated Paper Towel Filter Mats

Gelatin nanofibers were fabricated via facile electrospinning technique. A mono-inject syringe pump (KDS-100, KD Scientific), a plastic syringe (Monojet\textsuperscript{TM} Kendall) and a 21-gauge blunt tip needle were used to pump the gelatin solution. An operating voltage of 19-24 kV was applied and controlled using a high voltage power source (ES50P-5W, Gamma High Voltage Research) to draw the nanofibers.\textsuperscript{28} Paper towel substrate was fixed on a grounded commercial aluminum mesh with wire diameter of 0.011 inch and mesh pore size of 1 mm × 1 mm to collect the nanofibers onto the paper towel. The needle-collector distance was fixed to be 15 cm and a controlled feed rate of 0.5 ml/h was employed. During electrospinning, the horizontal and vertical position of the needle was continuously adjusted to deposit a uniform nanofiber mat with controlled diameter and thickness on the paper towel substrate.

### 4.2.3 Polluted Air Sample Preparation and Air Filtration Measurements

Tobacco smoke was selected to prepare the polluted air sample for testing. The details about the preparation of polluted air samples were described in previous chapters.\textsuperscript{27,28} The pressure drop (pressure difference between upstream and downstream of the filter) was measured by a differential pressure gauge (EM201-B, UEi) at different air face velocities. In addition, a portable air sampler (MiniVol Air Metrics, Eugene, OR, USA) was utilized to test the air filtration properties at different air flow rates. A circular filter sample with diameter of 37 mm was placed
in a home-made sample holder to perform air filtration testing. The filtered air was then collected in a clean plastic vacuum air-bag and similar measurements were carried out for the filtered air sample. To replicate the results, the testing process was executed on four composite filter mats prepared independently. One can calculate the removal efficiency ($\eta \%$) using the equation (1).

4.2.4 Characterization

Scanning electron microscopy (SEM, FEI SEM Quanta 200F) was used to study the morphology of the different paper towels and gelatin nanofiber-coated PT filter mats. All samples were coated with platinum nanolayer (3 nm in thickness) using Cressington high resolution sputter coater. In order to study the interaction-based filtration mechanisms, infrared (FTIR) transmittance spectra were collected on a Thermofisher iS10 spectrometer. The FTIR spectrum of pure SO$_2$ gas sample before and after filtration was utilized to study the toxic chemical filtration mechanism. Moreover, the mechanical properties of the paper towel and gelatin nanofabrics are tested using Instron machine 5969. All the measurement was repeated 4 times.

4.3 RESULTS AND DISCUSSION

As illustrated in Figure 4.1, we propose a novel method based on porous cellulose paper towel to address the materials and device level issues simultaneously. Paper towels are fibrous cellulose-based materials with good mechanical properties and high porosity. In this strategy, porous paper towels (PTs) are used as substrates, on which the protein nanofibers are directly deposited by electrospinning. It is expected that cellulose paper towel substrate will play three important roles: 1) it can mechanically reinforce the protein nanofabric and lead to good mechanical properties and
flexibility for the final filter (see the digital photo in Figure 4.1 and also Figure S3.1 and S3.2 in Appendix III); 2) it also can functionally contribute to the air filtration performance due to the porous structure and polar groups on the cellulose fibers; 3) paper towels with big pores can create a gradient porous structure for the final filter and act as a type of flow regulator to improve the interactions between pollutants and protein nanofabrics. Therefore, the incorporation of cellulose paper towel, as a disposable substrate for the protein nanofabrics, is a promising strategy to overcome the current issues of the air filters in both material and device level. The air filtration performance results will be discussed as follows.

**FIGURE 4.1.** Schematics of the preparation for the disposable paper-towel-based nano-air-filter with gradient pore sizes and functional surfaces. The protein nanofabric is deposited on either one side or both sides of a textured PT to control the air-filter configuration.
4.3.1 Structures and Air Filtration Performance of Paper Towels (PTs)

The porous structures and filtration performance of the paper towel substrate were investigated first. Three types of paper towels, including Scott®PT-T (textured surface), Scott®PT-P (plain surface), and Bounty®PT-B were investigated and the structures and performance were compared. In specific, the surface morphology/texture, porous structures, fiber areal density, fiber diameter and thickness were characterized (Please see Table S3.1, Figure S3.3 in Appendix III for detail information). The structures and air-filtration performance are shown in Figure 4.2. Based on the SEM images by Figure 4.2a-c, one can find that all the three types of PTs have cellulose fibers with similar diameters in the range of 10 to 13 μm (the fiber size distributions are shown in Figure S3.3 in the Appendix III), but different porous structures. The pore sizes and their distribution were determined via the ImageJ software for all three PTs (see Figure S3.4, Appendix III). It was found that the Bounty®PT-B possesses the smallest pore size of 22 μm, followed by the Scott®PT-P with an average pore size of 52 μm and the Scott®PT-T with an average pore size of 58 μm. At the same time, Bounty®PT-B and Scott®PT-T have a highly textured surface as compared with Scott®PT-P. Among these paper towels, the Bounty®PT-B also possesses the highest areal density, thickness and average fiber diameter. The two Scott® paper towels show similar numbers on those characteristics except the thickness.
FIGURE 4.2. Morphology and air-filtration performance of the three types of paper-towels. SEM images showing the surface morphology for (a) Scott®PT-T, (b) Scott®PT-P and (c) Bounty®PT-B (the insert are the digital photos of the paper towels). Air-filtration performance: (d) Air flow resistance (pressure drop), (e) Particulate removal efficiency and (f) PM$_{2.5}$ and PM$_{10-2.5}$ Particulate filtration efficiency, for the three types of paper towel.

The differences in structures, in particular, the pore structures, can significantly impact the air filtration properties and so the quality as a substrate for air-filtration. The filtration properties including particulate filtration efficiency ($\eta\%$) and the air flow resistance (known as pressure drop, $\Delta P$) of the three selected paper towels were tested. Pressure drop or the air flow resistance is one of the critical parameters related to the quality of the substrate for air-filtration application. For
paper towels, the Scott® PT-T gives rise to the lowest pressure drop (138 Pa) as compared with the other two as shown in Figure 4.2d. This is reasonable as Scott® PT-T possesses the biggest pores among the three types of PTs. In addition to the pressure drop, it is interesting found that even the PTs shows notable removal efficiency for particulate pollutants such as PM$_{0.3}$ (0.3 μm), PM$_{2.5}$ and PM$_{10-2.5}$, as shown in Figure 4.2e and f. However, it is noted that a high removal efficiency is realized only for big particulate pollutants such as PM$_{10-2.5}$, which is most likely filtrated by size-based capturing mechanisms.\textsuperscript{27,50} The above results indicate that porous PTs is not a high-performance air-filter material, but a good candidate as a flexible substrate which possesses a low air-flow resistance and even a high removing efficiency for big particles. The big pores not only provide a low air-flow resistance as shown above, but also create a gradient distribution of pore sizes when combined with a protein nanofabrics as proposed in Figure 4.1, which will be one of the important benefits from PTs as shown in this study. Considering the pressure drop, Scott® PT-T with the lowest air-flow resistance is selected as an example substrate for study in this chapter.

4.3.2 Gradient Nano-Air Filters (GNAFs) via Protein Nanofibers Coating onto PT

4.3.2.1 Morphology Studies of GNAF Before and After Filtration

The morphological characteristics of the pure gelatin nanofibers and the GNAF with gelatin nanofiber coated onto the paper towel (PT) were characterized before and after the filtration testing. The areal density of the protein materials for the pure gelatin nano-filter and the GNAF with the PT single-side coated by the gelatin nanofiber is fixed to be about 2 g/m$^2$ for comparison. Figure 4.3a and b illustrate the filter configuration and the definition of the front and back surfaces of the filter. The surface morphology before and after the filtration for the back and front surface were studied for the two situation as illustrated by Figure 4.3a and b. For pure gelatin nano-filter
before filtration testing, the back and front surface are the same since there is no pollutants absorbed (Figure S3.5, Appendix III). After the filtration, only the front surface facing the polluted air flow shows trapped pollutants while the back surface remains clean as shown by Figure 4.3c and d.

FIGURE 4.3. Morphology studies of the nano-filters before and after the filtration testing. (a) and (b): schematics of the filter configuration and the location for the definition of back and front surfaces. SEM images of the back (c) and front (d) surfaces for the pure gelatin nano-filter after the filtration testing; Digital photos of the GNAF before (e) and after (f) the filtration testing;
SEM images of the back (g and h) and front (i and j) surfaces for the GNAF with configuration of G/PT after the filtration testing.

However, the situation is very different when PTs is used as the substrate. Before filtration testing, there is no difference in the morphology for the gelatin nanofabric (G) on the PT (Figure S3.5, Appendix III). It is noted that the configuration of the GNAF sample with gelatin nanofabric facing the polluted air-flow during testing (see Figure 4.3b) is referred as G/PT. However, after the filtration testing, the surface morphology for both the front and back surfaces of the gelatin nanofabric is very different from the pure gelatin nano-filter as displayed by Figure 4.3g-j. From these SEM images, one can find that the GNAF with configuration of G/PT can capture a lot of particles/pollutants on the both sides of surface after exposure to the same polluted air for the same period of time. More significantly, it is found that almost all the nanofibers were coated by the pollutants when a paper towel substrate is employed (see Figure 4.3g–j and Figure S3.5, Appendix III). This significant finding indicates that the PT substrate can dramatically increase the chance for the pollutants to be absorbed by the protein nanofabrics, which will impact the removing efficiency a lot as shown later. The mechanism for this phenomenon will be analyzed in the last part of this study. At the same time, the digital photos in Figure 4.3e and f show clearly a color change from a milky color for fresh air filter (before filtration) to a yellow/brown color (after filtration). This color change indicates that both solid particles and gaseous chemicals were captured by the nanofibers from the polluted air. The morphology studies above indicate that the PT substrate can significantly change the filtration properties of the protein nanofabrics, which will be confirmed by the filtration performance as introduced below.
4.3.2.2 Air Filtration Performance

4.3.2.2.1 Filtration of Particulate Pollutants

In this chapter, we investigated the effects of the PT substrate on the particulate filtration properties of the GNAF. In particular, different filter configuration (see Figure 4.4a) for the combination of gelatin nanofabric (G) and the paper towel (PT) substrate has been studied. The filter configuration will change how the polluted air flow interact with the nano-filter and finally the air-filtration performance. To compare the performance, the amount of protein nanofabric used for the pure gelatin nano-filter and the GNAF with PT was kept the same to be around 2 g/m².

As shown in Figure 4.4b and c, there are two importing findings worthy of discussion. Firstly, by comparing with the pure gelatin nano-filter, the GNAFs with PT substrate can significantly improve the removal efficiency especially for small particulate pollutants with size less than ca. 1 μm. In particular, for particles with sizes smaller than ca. 1 μm, as shown in Figure 4.4c, the GNAF with PT substrate samples possess a removal efficiency of about 82.0% which is much higher than that of the neat gelatin (76.0%) for PM₀.₃. However, for particles with sizes larger than ca. 1 μm, the using of PT in GNAFs have no contribution to the efficiency, which lies in the range of 98.80 – 99.98%. At the same time, the performance of the PT substrate is included in Figure 4.4b for reference. Since the protein nanofabric used for them is the same, one can conclude that the PT substrate plays an important role in improving the efficiency particularly for small pollutants. These results can be explained by the fact that the removal of smaller particles with size less than the pore size is governed by an interaction-based mechanism between the pollutants and the nanofibers and, the using of PT can increase the possibility of the pollutants to interact with
the nanofibers, which has been confirmed by the SEM images (see Figure 4.3g-j). The mechanism for this enhancement will be analyzed later.

**FIGURE 4.4.** Particulate removal efficiency for the GNAFs with different filter configuration:
(a) Illustration of the GNAF with different filter configuration. (b) Particulate removal efficiency vs. various particle sizes (0.3-10μm) for the GNAFs as compared with the pure gelatin nano-
filter and PT; (c) enlargement of the removal efficiency for small PM particle sizes (0.3-2.5 μm); 
(d) Effects of PT and filter configuration on the PM$_{0.3}$ removal efficiency for the GNAFs; (e) 
Effect of filter configuration on the air flow resistance for the GNAFs.

Secondly, the configuration of the GNAF also plays a critical role in improving the removal 
efficiency as shown by Figure 4.4c and d. Among the three types of configuration as shown in 
Figure 4.4a, the GNAF with G/PT/G configuration shows the highest removal efficiency of 
99.30% for the smallest particulate pollutant of PM$_{0.3}$. At the same time, the GNAF with G/PT 
configuration shows a slightly higher removal efficiency for small particulate pollutions (PM$_{0.3}$ 
and PM$_{0.5}$) as compared with PT/G. This is a significant finding for practical application since one 
can remarkably improve the filtration performance by simply adjusting the filter configuration. It 
is believed that the filter configuration determines not only the distribution of functional surfaces 
for filtration, but also the pore configuration across the thickness direction of the filter, which is 
critical for the air flow inside the filter. To study this point, the air flow resistance for these filters 
were investigated at the same flow rate condition (4 L/min) and the results are shown in 
Figure 4.4e. From the air flow resistance, one can find that there is a good consistence between the 
removal efficiency and air flow resistance. In particular, the configuration with high flow 
resistance gives rise to a higher removal efficiency. This result is consistent with the fact that, for 
small pollutants, the filtration is based on interactions, and a high air resistance can increase the 
time for the pollutants to interact with the filter. Although the configuration can change the air 
flow resistance, the flow resistance for the highest one, the GNAF with G/PT/G configuration, is 
still well below the suggested limit of 325 Pa at a standard air flow rate of 4 L/min., by DOE.$^{25}$
4.3.2.2 Filtration of Toxic Chemicals

In addition to improve the particulate filtration performance, the GNAFs with the PT substrate also significantly improve the filtration efficiency for multiple types of toxic chemicals as compared with pure gelatin nano-filter. In this chapter, four types of toxic gases with different molecular structures, such as formaldehyde (HCHO), carbon monoxide (CO), sulfur dioxide (SO₂), and volatile organic compounds (VOCs), were chosen to test the toxic chemical removal efficiency. These chemicals are carcinogens and very toxic gases that are present at the hazardous level in cigarette smoke. The test results are summarized in Table 4.1. Similar to the findings for particulate pollutants, it can be found that the use of PT substrate can significantly increase the removal efficiency for all the four types of chemicals as listed in Table 4.1.

TABLE 4.1. Filtration performance for various toxic chemicals

<table>
<thead>
<tr>
<th>Sample</th>
<th>EHCHO (%)</th>
<th>ECO (%)</th>
<th>ES02 (%)</th>
<th>EVOCs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>13.30±3.24</td>
<td>20.70±2.48</td>
<td>11.40±2.20</td>
<td>27.55±1.50</td>
</tr>
<tr>
<td>Gelatin</td>
<td>65.00±1.24</td>
<td>62.34±1.38</td>
<td>63.38±1.12</td>
<td>79.14±1.20</td>
</tr>
<tr>
<td>Gelatin/PT</td>
<td>77.37±0.98</td>
<td>69.32±1.10</td>
<td>79.85±0.80</td>
<td>82.82±1.30</td>
</tr>
<tr>
<td>PT/Gelatin</td>
<td>76.92±1.40</td>
<td>69.26±0.75</td>
<td>77.94±0.52</td>
<td>80.68±0.33</td>
</tr>
<tr>
<td>Gelatin/PT/Gelatin</td>
<td>83.70±1.10</td>
<td>80.22±1.40</td>
<td>81.77±0.46</td>
<td>83.70±0.55</td>
</tr>
</tbody>
</table>

In specific, for the GNAFs with different configuration, the efficiency is improved by more than 10% for most of the chemicals as compared with the pure protein nano-filter. As a reference sample, the PT shows the lowest removal efficiency below 20% for most of the chemicals. In
addition, the configuration also plays a critical role in improving the removal efficiency for the toxic chemicals. In particular, the GNAF with G/PT/G configuration shows the highest efficiency for all the chemicals as compared with the other two GNAFs. Since chemicals are also small pollutants with size much smaller than the pore size of the filter, the filtration performance is similar to the small particulate pollutants as shown in Figure 4.4. For the specific interactions between the toxic chemicals and the nano-filter, one can find these information in previous chapters. In particular, we have found that the active aldehyde group of HCHO can interact with the functional groups of gelatin forming a strong chemical bond responsible for the higher removal efficiency of HCHO; For CO, it can interact with the gelatin molecules via a polar-polar interaction since it is a polar molecule. These results indicate that the combination of the gelatin nanofibers and PT substrate can dramatically increase the removal capability for small pollutions with size below the pore size of the filter.

FIGURE 4.5. Study the removal of SO$_2$ by GNAF with PT substrate. (a) Schematic of the apparatus used for the IR measurements; (b) IR spectra of the air sample before and after
filtration of SO$_2$ gas by a PT filter; (c) IR spectra of the air sample before and after filtration of SO$_2$ gas by a GNAF with G/PT configuration.

In addition to the studies on the removing efficiency of toxic chemicals by chemical sensor, we particularly investigated the removing of SO$_2$ by FTIR in this chapter. As shown in Table 4.1, an excellent SO$_2$ removal efficiency (77.9 to 81.8\%) has been achieved for the GNAFs, which is much higher than that of neat gelatin nano-filter (about 63.38\%). To test the removal ability for SO$_2$, the filter sample was placed inside a special designed set-up as shown in Figure 4.5a. For comparison, the IR spectra of SO$_2$ before and after the filtration was collected and compared for two kinds of samples: the PT substrate and the GNAF (protein nanofabric coated PT). The results are shown in Figure 4.5b and c, one can find that the PT substrate shows a negligible contribution to the removal of SO$_2$, while, the GNAF possesses a high removal efficiency for SO$_2$ due to the combination of PT and protein nano-fabrics. The high removal efficiency of toxic chemicals by the protein/cellulose filters is mostly due to enhanced intermolecular interaction-based mechanisms.$^{27,28}$ These mechanisms involve primarily the interaction of the functional groups existing in the molecular structures of gelatin (protein) and cellulose, since the gaseous pollutants are very small molecules and cannot be removed via the primary physical mechanisms,$^{50}$ which is only effective for the filtration of particulate pollutants.

4.3.2.2.3 Filtration Performance vs. Flow Rate

Additionally, the effect of air flow rate on the filtration performance of the GNAF with G/PT/G configuration was further investigated in this chapter. The results are shown in Figure 4.6. Firstly, for the particulate pollutants, it can be found that the effects of flow rate on the removal efficiency
is highly dependent on the size of the pollutants. In particular, for big particles with size larger than the pore size of the filter, there is no flow-rate dependent behavior since they are filtrated by sized-based mechanism which is not affected by the air flow rate. However, for small pollutants with size lower than the pore size, the smaller the size is, the stronger the dependent behavior will be. This result confirms that the mechanisms for the filtration of small pollutants is interaction-based, which is sensitive to the flow rate. Secondly, for the chemical pollutants, there is also a flow-rate dependent behavior for different types of toxic chemicals. This result is consistent with the situation for the small pollutants as described above since the filtration mechanisms for both of them are similar. However, it is noted that the flow-rate dependent behavior for SO₂ and VOCs is slightly weaker than that for other two chemicals, indicating that the nano-filter may have stronger interactions with SO₂ and VOCs as compared with the other two. Although the efficiency is dependent on the flow-rate, overall it maintains a high value when the flow rate increases from 4 to 10 L/min. In specific, the efficiency for small particulate pollutants is still higher than 98% even for the smallest one PM₀.₃. While, it is above 75% for all the toxic gases.

FIGURE 4.6. Effects of air flow rate on the filtration performance for the GNAF with G/PT/G configuration. (a) Effects of flow rate on the removal efficiency for particulate pollutions with
small sizes from 0.3 to 2.5 μm. (b) Effects of flow rate on the removal efficiency for chemical pollutions. (c) Air flow resistance vs. flow rate (inserted image is the schematic illustration of the pressure drop measurement apparatus).

It is noted that the air-filtration performance should also take the flow resistance (or pressure drop) into account since it is related to the energy consumption of the filter. For a high filtration efficiency filter, such as HEPA grade filters, it must possess a pressure drop of less than 325 Pa at a standard air face velocity of 5 cm/s.\textsuperscript{25} As shown in Figure 4.4e, we have demonstrated that the filter configuration plays an important role in controlling the air flow resistance since it is related to the pore configuration of the filter. Here, we further investigated how the flow rate affects the air flow resistance of the GNAF with G/PT/G configuration which shows the best filtration performance as shown above. As shown in Figure 4.6c, the pressure drop increases only slightly from 168 Pa to 195 Pa as the air flow rate rises from 4 to 10 L/min, which is well below the value of 325 Pa suggested by DOE. This increase can be due to the change in the air stream pathways when it passes through the multiple layers of nanometer and micrometer size fibers. In sum, the GNAF with protein nanofabric layers coated onto a paper towel can significantly improve the filtration performance (see Figure 4.4, Table 4.1 and Figure 4.5), while keeping the pressure drop below the DOE standard. The above results indicate that the protein/PT based gradient nano-air-filters (GNAFs) are promising as a high performance “green” multifunctional air filters to be utilized in various air filtration applications.
4.3.2.3 Mechanism Analysis

To understand and explain the outstanding filtration performance of the GNAF with protein nanofiber-coated onto PT substrate, we further analyze the possible mechanism for the PT contribution to the filtration performance. It is noted that the filtration mechanisms for the protein nano-filter were analyzed and studied in previous chapters. Therefore, this section of this chapter is focused on the mechanisms on how the PT substrate can improve the filtration performance as compared with the pure protein nano-filter. As shown in Figure 4.7a, first of all, it is believed that the use of PT substrate can significantly change the air-flow streams, which can impact the performance. In this study, the textured PT may cause some circulatory flow at the interface between the protein nanofabric and the PT substrate as illustrated. These circular flows enhance the chance for the pollutants (especially the small pollutions with size much lower than the pore size of the filter) to interact with the protein nanofabric. This picture can explain well the morphology results as shown in Figure 4.3i and j, which clearly shows that the use of PT substrate can significantly increase the chance for the protein nanofabrics to interact with the pollutants and almost all the nanofibers on both sides were covered by pollutants after the filtration.
FIGURE 4.7. Schematic of the contribution to filtration performance from PT. (a) Comparison of the situation without and with PT substrate: the one without PT showing single-side absorption of pollutions via interaction-based filtration; the one with PT showing double-side absorption due to regulated air flow by the PT. Schematic of the filter configuration with different pore distribution and flow process: (b) - (d): the PT/G, G/PT and G/PT/G nanofilters with gradient pore distribution due to the combination of protein nano-fabrics and PT cellulose micro-fibers. The use of PT and filter configuration can contribute to the air flow and greatly impact the filter-pollutions interactions.

In addition to a textured surface, the layered configuration of the combined nano-filter with protein nanofabric and cellulose micro-fibers lead to special porous structures (see Figure S3.6, Appendix III). In particular, the combination of the small pores of the protein nanofabric (ca. 4.5 μm) and
the big pores of the PT generates gradient porous structures for the resultant nano-filter, which can affect the air-flow fundamentally. Figure 4.7b–d illustrate the air flow and pore structures for the filters with different configuration. The difference in the pore structures of the layered filter can explain the air flow resistance and the filtration performance as shown previously. For example, for the GNAF with PT/G configuration (see Figure 4.7b), the PT with big pores faces the air flow and few or weak circular flow will be generated since the nanofabric possesses a more uniform and smaller pore size. At the same time, even there is strong circular flow, the circular flow won’t notably contribute to the filtration performance since the flow goes back to the PT, not the protein nanofabrics which is the key to absorb the small pollutants. The situation will be different for the G/PT configuration as illustrated in Figure 4.7c. In this case, more circular flow will be generated due to the textured surface of PT as well as the big fibers of PT. At the same time, the circular flow will contribute the filtration performance since the flow goes back to the nanofabrics. For G/PT/G configuration, it maximizes the performance due to the two layers of protein nanofabrics on both side of the PT. In short, the use of PT substrate and the filter configuration can significantly change the air flow streams and so the chance for the pollutants to be absorbed by the protein nanofabrics.

4.4 CONCLUSIONS

In this chapter, we have demonstrated that the common paper towel can be engineered with protein nanofabrics and thus a complete disposable air-filter with very high filtration efficiencies for both particulate and toxic gaseous chemical pollutants can be achieved. The protein nano-filter is significantly enhanced mechanically and functionally by incorporating the paper towel substrate. Due to the special gradient porous structures of the layered filter and the presence of multiple functional groups in the protein structure, the resultant filter is capable of interacting with various
pollutants and gives rise to improved removal efficiencies for particularly small pollutants with the size less than the pore size of the filter, as compared with pure protein nanofiber-filter. The studies in this chapter indicate that the porous structures and mechanical flexibility of the common paper towel can provide significant advantages for fabrication of high performance air-filters. Moreover, since all the materials involved are environmentally friendly and extremely abundant, the reported air-filter can be used as a disposable device in practice. This is important as it does not only address the disposal issues of air-filter materials, but also provides a simple solution for the fast deterioration of air-filters when highly polluted air is present.
CHAPTER FIVE: A CROSS-LINKED PROTEIN NANO-FILTER WITH ANTIBACTERIAL PROPERTIES FOR MULTIFUNCTIONAL AIR FILTRATION

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ABSTRACT: Development of high-performance nanomaterials with not only strong ability to trap the pollutants, but also good structure stability under varying environmental conditions is in critical need for air-filtration applications. However, it has been very challenging for a filtering material to simultaneously realize multifunctional air filtration and good environmental stability. In this chapter, based on our studies presented in previous chapters on protein-based nano-filters, we report a cross-linked protein nanofabric to address this challenging issue. It is found that cross-linked protein nanofabrics can significantly improve the structure stability against different moisture levels and temperatures, while maintain the multi-functional filtration performance. Moreover, it is demonstrated that the cross-linked protein nanomaterials also possess antibacterial
properties, such as *Shewanella oneidensis* and *Staphylococcus aureus* bacteria, further improving the environmental stability. The effects of cross-linking with different loading of cross-link agent on the structure stability and filtration performance are further investigated at different humidity levels and temperatures. This chapter provides a cost-effective solution for advanced “green” nanomaterials with excellent performance in both filtration functions and structure stability under varying environment.
5.1 INTRODUCTION

Airborne contaminants such as particulate matter (PM) of various sizes, chemical mixtures, and microbiological particulates, have increasingly become concerns in defining air quality, especially the indoor air quality, in the past decade. The particulate pollutants are usually categorized into different groups according to the size, such as the well-known PM\(_{2.5}\) with sizes less than 2.5 μm and PM\(_{10-2.5}\) with sizes in the range of 2.5 – 10 μm. Additionally, polluted air also includes various types of toxic gaseous chemicals such as nitrogen oxides (NO\(_x\)), sulfur oxides (SO\(_x\)), formaldehyde (HCHO), carbon oxides (CO and CO\(_2\)), and combination of other volatile organic compounds (VOCs). Moreover, bio-aerosols such as bacteria, viruses, and fungi represent another type of primary pollutant which greatly raise up the risks on the public health since they can easily spread over by polluted air very quickly. These biological pollutants can cause both chronic and acute diseases that can be allergenic, infectious, toxigenic and etc. This was proved by the significant discovery of the Severe Acute Respiratory Syndrome (SARS) and Middle Eastern Respiratory Syndrome (MERS). Due to the complicated biophysicochemical interactions between human body and pollutants in the air, it is believed that more and more potential risks for health will be discovered in the near future. Therefore, development of advanced nanomaterial for air-filtration is in critical need by public health.

Due to the complicated compositions in polluted air and varying working conditions of air-filters, it is critical for air-filtering materials to achieve high-performance in both multiple filtration and structural/function stability under varying environmental conditions. However, realization of this kind of high-performance air-filtering materials has been challenging for the following reasons. Firstly, conventional fibers employed for air-filters are petroleum-derived polymers (such as
polypropylene) which show excellent environmental stability with moisture, chemicals and temperatures. However, there are two major drawbacks for these conventional polymer-based air filter materials. The first one is that the filtration function is highly limited by the size-based mechanism and few types of functional group can be available to trap the pollutants. In other words, the fiber surface is poorly functionalized to trap pollutants, which significantly affect the filtration performance especially for pollutants with size much smaller than the pore size. The second drawback comes from its excellent stability. It is well-known that petroleum-derived polymers are so stable that they cannot degrade naturally, which causes serious environmental issues after disposal. To address these materials issues, we have developed “green” multifunctional air filtering materials made of natural protein nanofibers.\textsuperscript{27,28} Owing to the in-built functional groups from about 20 types of amino acids, the natural protein nanofibers showed high affinity toward various types of pollutants, which resulted in excellent removal efficiencies for both particulate pollutants, and various species of toxic chemical gases. Although the materials issue in filtration functions has been well-solved, this protein nanofabric was mechanically weak, and importantly, demonstrated poor environmental stability toward moisturizes, which raises a big concern for its practical applications. Therefore, for the protein-based nano-filters developed in our lab, improving the environmental stability is a critical step toward their broad applications.

Crosslinking has been widely employed to improve the structural stability and water resistance of polymers in materials science. In particular, for proteins, there are many methods proposed to crosslink protein molecules, such as dehyrothermal treatment,\textsuperscript{211} ultraviolet (UV) treatment,\textsuperscript{212,213} and utilization of crosslinking agents. In general, physical treatments result in low crosslinking degree since the crosslinking reaction induced by UV is limited near the surface of the material.\textsuperscript{211}
Crosslinking agents represent the most important and primary way as it can generate controlled degree of cross-linking via usually simple and efficient procedures. As a result, various kinds of crosslinking agents have been used for protein cross-linking, such as glutaraldehyde (GTA)\textsuperscript{214,215}, formaldehyde, genipin\textsuperscript{216}, 1-ethyl-3-(3-(dimethylaminopropyl)-carbodiimide hydrochloride (EDC)\textsuperscript{216} etc. Recently, carbodiimide crosslinking agent was also reported as a green crosslinking agent for proteins. However, to achieve an appropriate crosslinking degree for enhancement of water resistance and mechanical properties, N-hydroxy succinimide (NHS) must be added as an additive for the carbodiimide crosslinking agent\textsuperscript{216–218} which not only increases materials cost, but also make the crosslinking reaction more difficult to control\textsuperscript{216}. Among all of these chemical crosslinking treatments, GTA vapor treatment has been most widely used in particular for gelatin protein crosslinking\textsuperscript{214,215}. However, GTA is also costly, and more seriously, a very toxic chemical\textsuperscript{219,220}. In brief, searching “green” cross-linking agents which work well for particularly proteins and is compatible with electrospinning process is the key to fabricate environmentally stable protein nanofabrics.

Moreover, to achieve good environmental stability for protein-based filters, the effects of bacteria on the structural stability of the filter should also be considered. As it is mentioned above, bioaerosols are one of the most hazardous pollutants threatening the human health, thus, a high-performance air filter is expected to not only capture the biohazards, but also to kill the biohazards. However, accumulation of the captured bioaerosols on air filters raises a critical issue as they can grow and spread on the filter in presence of sufficient moisture and nutrients\textsuperscript{41,42,44}. Moreover, the particulate and chemical pollutants captured on the filter can further contribute to the growth of the bioaerosols which may lead to a significant decrease in filter’s efficiency and eventually
deterioration of the filter (bioporation). Additionally, the volatile chemicals produced from the microbial metabolism can also be released from the damaged filters to the air again.\textsuperscript{41,43} Such phenomenon have led to outbreaks of Legionnaires’ disease and Pontiac fever.\textsuperscript{221–224} In other words, used filters can be a source of secondary pollutants and detrimental to the air quality and human health. Conventionally, antibacterial properties are realized by treating or mixing fibrous filters with different conventional antimicrobial agents such as heavy metals (e.g. silver nanoparticles),\textsuperscript{45,46} metal oxides (e.g. Titania (TiO2), zinc oxide (ZnO) and copper oxide (CuO)),\textsuperscript{47,48} carbon nanotube (CNT),\textsuperscript{225,226} iodine,\textsuperscript{227} small molecule biocides. Recently, the extracts of natural products such as \textit{Saphora Flavescens} Ait was also reported as an effective solution to address this issue.\textsuperscript{44,228} Therefore, achieving antibacterial properties for our protein-based air nanofilter is critical for its long-term performance and safety.

In this chapter, via an appropriate choice of non-toxic cross-linking agent, a cross-linked protein nanofabric is successfully fabricated via electrospinning for air filtration application. The effects of humidity levels, temperature on the filtration performance of the un-crosslinked and crosslinked gelatin/paper towel (G/PT) hybrid filters were investigated in details. Moreover, the stability of the cross-linked protein-nanofiber mats with biological pollutants, a gram-negative bacterium called \textit{Shewanella oneidensis} and a gram-positive bacterium called \textit{Staphylococcus aureus}, was further studied via the inhibition zone method.
5.2 EXPERIMENTAL

5.2.1 Raw Materials and Solution Preparation

Gelatin powder (type A, from porcine skin) was supplied from Sigma Aldrich (MO, USA). Glacial acetic acid (AcOH) with 99.9% purity was purchased from J.T.Baker® (PA, USA). The carbodiimide crosslinking agent was purchased from Sigma. Gelatin solution was prepared in a mixture of AcOH : DI water with a 80:20 volume ratio following the study in chapter one. Then, the crosslinking agent was mixed with the solution with a loading from 5-20 wt%. The mixture solutions were stirred at room temperature at 400 rpm to achieve homogenous solutions.

5.2.2 Preparation of the Crosslinked Gelatin-Based Hybrid Air Filter Mats

The gelatin solutions containing different loadings of crosslinking agent (from 0 to 30%) were fabricated into nanofiber mats using the electrospinning technique. A mono-inject syringe pump (KDS-100, KD Scientific), a plastic syringe (Monoject™ Kendall) were utilized to pump the solution from a 21-gauge blunt—tip needle. An operating voltage of 15-17 kV was applied for the gelatin solution using a high voltage power source (ES50P-5W, Gamma High Voltage Research) to fabricate the nanofabric mats. A commercial Scott® paper towel was used as a porous substrate fixed on the aluminum mesh grid with the mesh pore size of 1 mm × 1 mm and wire diameter of 0.011 inch. Aluminum grid was fixed and grounded to collect the nanofabrics. The needle-to-collector distance was secured to be 20 cm. The feed rate of 0.6 mL/h was employed and the vertical and horizontal location of the needle was constantly tuned to deposit a uniform mats with controlled thickness, areal density, and fiber diameter.
5.2.3 Antimicrobial Property Testing

The antimicrobial activity of the paper towel (as the control sample) and crosslinked gelatin-based filter material was determined by the Kirby-Bauer (inhibition zone test) qualitative method. For this purpose, *Shewanella oneidensis* MR-1 and *Staphylococcus aureus* were utilized as gram-negative and gram-positive testing microorganisms. Before the experiment, all the materials except filter materials were autoclaved at 120 °C for 15 min. To sterilize the filter materials, each side of the samples were exposed to UV for 15 min. *S. oneidensis* cells were cultivated in sterilized Luria-Bertani (LB) broth medium and incubated at 28 °C in a shaking flask in an incubator for 24 hours. *S. aureus* cells were cultured in sterilized tryptic soy broth (TSB) (Fisher Scientific, catalog #211825) at 37 °C on a rotary shaker (Lab LineL. E. D. Orbital Shaker Model 3518, 90 rpm) to prepare an overnight culture. To perform antimicrobial testing, the sterilized circular-shape filter samples with a diameter of 1 cm were placed on the LB agar plate which was covered with *S. oneidensis* cells containing 3.5×10^6 colony forming units (CFU/ml) and incubated for 24 hours at 28 °C. The same procedure was applied for *S. aureus* except that instead of LB agar plates, TSA agar plates were used to test the antimicrobial property. The biocidal activity of the filter samples was identified and estimated by an inhibition zone around the samples. All tests were performed at least four times to ensure biological reproducibility.

5.2.4 Characterization and Air Filtration Testing

Scanning electron microscopy (SEM, FEI SEM Quanta 200F) was utilized to investigate the morphological characteristics of the un-crosslinked and crosslinked gelatin nanofabric filter mats. All the filter samples were first coated with a gold nanolayer (10 nm thick) via a Technics Hummer V sputter coater. Fourier transform infrared (FTIR, Thermofisher iS10) spectroscopy was
employed to study the functional groups before and after crosslinking. Additionally, differential scanning calorimetry (DSC, Mettler Toledo 3+) was utilized to study the effects of crosslinking on the thermal properties of the gelatin nanofabrics. The air-filtration testing procedures were described in previous chapters.

5.3 RESULTS AND DISCUSSION

5.3.1 Crosslinking of the Gelatin Nanofabrics

In order to increase the humidity and temperature stability of proteins, the gelatin nanofibers are crosslinked with a crosslinking agent. In this section, the structure of the crosslinked gelatin is first studied. Figure 5.1a illustrates the possible crosslinking mechanism of the gelatin molecules. It is well-known that the gelatin molecules possess numerous functional groups including carboxylic and amine groups. For the un-crosslinked gelatin molecules, only intra-molecular secondary bonds including hydrogen bonding and Van der Waals interactions as well as physical entanglements present among the gelatin molecules. However, after crosslinking, the crosslinking agent chemically react with amino or carboxylic groups, which leads to the formation of intra-molecular and intermolecular crosslinks, respectively.\textsuperscript{216,229,230} We studied the crosslinking of the gelatin nanofibers via Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC). Figure 5.1b displays the FTIR spectra of the un-crosslinked and crosslinked gelatin nanofabrics. It was observed that these two samples possess peaks at 1650 cm\textsuperscript{-1} (amide I), 1536 cm\textsuperscript{-1} (amide II), and 1236 cm\textsuperscript{-1} (amide III).\textsuperscript{231–233} Some studies reported that the electrospinning process can convert the gelatin molecules from a less-ordered β-sheets to a more regular and ordered α-helix structure.\textsuperscript{232} Interestingly, the FTIR result shows that, for the crosslinked gelatin, the intensity of the amid I band increased and the peak shifted from 1650 cm\textsuperscript{-1} to
1644 cm⁻¹, which means crosslinking may help to form or maintain more α-helix structure. At the same time, the increase in intensity may be related to the formation of amide bonds as the result of the crosslinking. Additionally, the DSC results shown in Figure 5.1c exhibit that the transformation (Tᵣ) of the gelatin nanofibers has been mostly suppressed after crosslinking, which is consistent with the picture that cross-link can remarkably reduce the mobility of gelatin chains and so the thermal transformation process. Based on the above FTIR and DSC results, one can conclude that the gelatin nanofibers were successfully crosslinked.

FIGURE 5.1. (a) Schematic illustration of the crosslinking for gelatin molecules; (b) FTIR spectra of the un-crosslinked and crosslinked gelatin nanofabrics; (c) DSC curve of the un-crosslinked and crosslinked gelatin nanofabrics at 10 °C/min heating rate.
5.3.2 Effects of Crosslinking Agent Loading on Gelatin Nanofabric Morphology

In this section of the chapter, we first investigated the effect of the crosslinking agent loading on the morphology of the gelatin nanofabrics. The particulate and chemical air filtration performance and the pressure drop of these samples were then tested. To be comparable, the areal density of the gelatin nanofiber in the samples was fixed to be about 2 g/m². Figure 5.2a–f illustrate the morphology of the un-crosslinked gelatin nanofabric and cross-linked gelatin nanofabrics with various loadings of the crosslinking agent before the filtration testing. From the SEM images, one can find that the addition of crosslinking agent can increase the fiber diameter from 80 to ca. 270 nm. The fiber diameter distribution for each sample is shown as inserts in Figure 5.2. It is noted that the diameter of the spun fibers increased from 87 nm to 207 nm after adding 5% of crosslinking agent, and the samples with 10 and 15% loadings of the crosslinking agent resulted in uniform fiber mats and only slightly increase in the fiber size to be 215 and 245 nm, respectively. Moreover, the fiber diameter slightly increased to about 261 nm for the sample with addition of 20% crosslinking agent. It was also observed that the sample with 30% loading of crosslinking agent possessed very poor electrospinability and resulted in a non-homogenous fibrous film (see Figure 5.2f; see Figure S4.1 for more SEM images, Appendix IV). These results showed that the addition of the crosslinking agent leads to a noticeable increase in the fiber diameter; however, the fiber diameter of the samples did not significantly change with the loading of crosslinking agent above ca. 10 wt%. In addition to the fiber diameter, pore size is another critical factor affecting the air filtration performance of an air filter. As it is challenging to determine the pore geometry of the nanofiber mats due to the irregular shape of the pores, we estimated the pore size of the samples via Image J software.27 The results show that the pore size distribution of these un-crosslinked and
crosslinked gelatin nanofibers is similar and the average size is around 3.9 μm (also see Figure S4.2, Appendix IV).

![SEM images of gelatin nanofabrics with different crosslinking agent loadings](image)

**FIGURE 5.2.** Microstructures studied by SEM images for the gelatin nanofabrics with different loading of the crosslinking agent: (a) 0%, (b) 5%, (c) 10%, (d) 15%, (e) 20%, and (f) 30%. The scale bar is 1 μm. The inserts are the fiber diameter distribution of the nanofibers.

### 5.3.3 Filtration Performance at Different Humidity Levels

In order to study how the humidity level affects the structures and filtration performance of the crosslinked gelatin nanofabrics, the minimum loading of crosslinking agent to generate sufficient moisture resistance was first investigated. To do that, the filtration performance and
microstructures of the filters after filtration at a high level of humidity was studied. Figure 5.3a-c show the particulate, toxic chemical filtration efficiency and the pressure drop for the samples with different loading of cross-linking agent at 95% humidity. At the same time, the microstructures of these samples after filtration testing were characterized by SEM as shown in Figure 5.3d-g. From Figure 5.3a and b, one can find that the particulate removal efficiency at a very high moisture content (95% RH) for particularly small particles with sizes less than 2.5 μm is dependent on the loading of the crosslinking agent. The filter samples with no or low cross-linking agent (lower than ca. 5 wt%) show much lower removing efficiency for both particulate and chemical pollutions. While the samples with loading of 10 wt% or 15 wt% show much better performance at this high humidity level. To understand the above results, we further performed pressure drop testing at this humidity level and the results are shown in Figure 5.3c. It can be easily found that the pressure drop is very sensitive to the loading of crosslinking agent. An insufficient loading of the crosslinking agent gave rise to much higher pressure drop. When the loading is above 10 wt%, a stable pressure drop was observed. However it is noted, for the sample with 20 wt% of crosslinking agent, a much lower pressure drop was recorded, which is mainly due to the formation of cracks (see the insert in Figure 5.3c). The formation of cracks is due to the fact that a high crosslinking degree will lead to increased brittleness and mechanical weakness. The pressure drop testing indicates that, to achieve stable structures under the high humidity level, there is an optimum loading of cross-linking agent.

To further understand the pressure drop and filtration performance, the microstructures of the samples after filtration testing were studied by SEM images. From Figure 5.3d and e, one can find that the sample with no crosslinking agent was mostly dissolved and formed a solid transparent
film after exposure to high humidity air (see the insert photo). Similarly the sample with 5 wt% loading of crosslinking agent was also destroyed somehow. The dissolving of nanofibers finally resulted in a non-porous film and cracks under air-flow (also shown in Figure 5.3d and e) due to a high pressure drop. These results can explain the above finding that the sample with no or low loading of crosslinking agent led to very low removal efficiency for both particulate and chemical pollutions, and at the same time, a high pressure drop. In comparison, the samples with 10-15 wt% loadings of crosslinking agent showed significantly improved structure stability at 95% humidity as shown in Figure 5.3f and g. The good structure stability with humidity explains the high filtration efficiency and low/stable pressure drop as shown in Figure 5.3a–c. Based on the above results, one can conclude that there is an optimal loading of crosslinking agent which can effectively improve the moisture resistance property of the nanofibers and, in the meantime, maintain the mechanical flexibility and the air filtration performance of the filters. To minimize the crosslinking loading, 10 wt% was chosen as an optimized loading of crosslinking agent for the further testing in the following studies in this chapter.
FIGURE 5.3. Determining the optimum loading of cross-linking agent for the gelatin nanofabrics at a high humidity level of 95%. (a) particulate removal efficiency vs. particle sizes (0.3-10μm) for the samples with different cross-linking agent loading; (b) chemical removal efficiency for the two toxic gaseous molecules (formaldehyde (HCHO) and carbon monoxide (CO)); (c) air flow resistance; SEM images of filters after testing with crosslinking agent loadings of (d) 0%, (e) 5%, (f) 10%, (g) 15%. The scale bar is 1 μm. The inserts are the digital photos of the filters after filtration testing.

The particulate and chemical removal efficiency of the crosslinked Gelatin nanofibers/PT hybrid filters with the optimal loading of the crosslinking agent (10%) at different humidity levels were further studied and compared with that of the hybrid filters with no crosslinking. The filtration properties include those of small solid particles and the two toxic gaseous chemicals as shown
previously. Figure 5.4a–c show the particulate removal efficiency comparison of the filters with crosslinked and un-crosslinked gelatin nanofiber mats for PM with different sizes: including PM$_{0.3}$, PM$_{0.5}$, and PM$_{2.5}$ at different humidity levels, respectively. The filtration performance for these particle sizes was investigated because the removal of smaller particles is more challenging as they are much smaller than the pore size of the filter. From the Figure 5.4a and b, it can be seen that the un-crosslinked filter possesses slightly higher removal efficiency for PM$_{0.3}$ and PM$_{0.5}$ than that of the crosslinked filter at low humidity levels. This is possibly due to the fact that the un-crosslinked gelatin nanofibers have smaller fiber diameter (about 87 nm) as compared with that of the crosslinked filters (about 250 nm). As demonstrated in previous chapters, the filtration of small particles are mostly governed by the interaction-based capturing mechanisms, which is dependent on the surface chemistry as well as surface area. In addition, by crosslinking the protein molecules, the density of free active groups to interact with the pollutants may be reduced after crosslinking.

However, for the un-crosslinked sample, the filtration efficiency of the small particles decreased a lot at the 60% relative humidity and then increased as the humidity level increased to 75%. Although this phenomenon is not fully understood, we believe that, at a certain point above 60% RH, the water molecules absorbed by the filter can help to capture pollutants which result in increased filtration removal efficiency. On the other hand, Figure 5.4c shows that for larger particles (PM$_{2.5}$) the removal efficiency of the crosslinked sample did not change at almost all testing humidity levels. Since the porous structure of the un-crosslinked nanofiber sample was destroyed and a gelatin film was formed at 95% humidity, no reliable data was collected for the un-crosslinked sample at this humidity level. The morphological characteristics of these filters after filtration at different humidity levels also show that the un-crosslinked sample can maintain
their porous structures up to humidity level of ca. 70% RH (see Figure S4.3, Appendix IV). While the crosslinked sample possessed removal efficiency of 98.61, 99.18, and 99.78% for PM$_{0.3}$, PM$_{0.5}$, and PM$_{2.5}$, respectively, at high humidity level. In short, the crosslinking significantly improves the performance stability for particulate removal at different humidity levels from 25% to 95%, which is critical for its practical applications.

FIGURE 5.4. Effects of humidity levels on the filtration performance of the crosslinked gelatin nanofabrics as compared with the un-crosslinked nanofibers: Particulate removal efficiency for (a) PM$_{0.3}$, (b) PM$_{0.5}$, and (c) PM$_{2.5}$. Toxic chemical removal efficiency for (d) formaldehyde and (e) carbon monoxide.
Similarly, we further investigated the effects of humidity and crosslinking on the removing efficiency for toxic chemicals (e.g. HCHO and CO). For the chemical removing, there are two important findings worthy of discussion. The first one is that humidity level plays an important role in controlling the removing efficiency for some specific chemicals. For example, as shown in Figure 5.4d, for both the un-crosslinked and crosslinked samples, the HCHO removal efficiency increased notably from 70% to about 80% when the humidity increases from 25% to 75%. However, for CO, there is no obvious change but only slight decrease in the efficiency with the increasing of humidity level as shown in Figure 5.4e. The second important finding is that the crosslinking also affects the chemical removing efficiency, but differently for different chemicals. More specifically, it can reduce the removing efficiency for both HCHO and CO, but more dramatically for CO. The above result can be explained by the effects of cross-linking on the water absorption ability as discussed below. First of all, it is well-known that HCHO is highly soluble in water (about 400 g/L). Therefore, with the increase in humidity level, more moisture molecules will be absorbed by the gelatin nanofibers, and thus, more formaldehyde molecules will be dissolved in the gelatin nanofabric, which results in the increase in HCHO removal efficiency. For the same reason, the effects of humidity level for CO removing is not as strong as that for HCHO since the solubility of CO gas in water is much lower (about 27 g/L). For the effects of crosslinking on the chemical removing efficiency, one can understand it from two aspects. Firstly, it works together with the moisture to influence the efficiency. More specifically, the crosslinking can reduce the ability to absorb moisture, which finally reduces the removal efficiency since water can help to absorb more water-soluble pollutants. Secondly, as analyzed in previous chapters, the chemical removal ability of the protein-based filtering materials is governed by interaction-based capturing mechanisms. These interaction-based mechanisms are provided by the interactions
between the functional groups existing in the protein structure and the pollutants. It is noted that the crosslinking process can consume part of the functional groups (e.g. carboxylic and amine groups), resulting in less available groups to interact with the pollutants.

5.3.4 Filtration Performance at Different Temperatures

In addition to humidity, temperature is another critical environmental factor that can impact the filtration performance in practical applications. Therefore, the effects of temperature on the particulate and toxic chemical filtration performance for the crosslinked and un-crosslinked protein-based hybrid filters were investigated in this chapter. For this purpose, a practical temperature range between 25°C to 45 °C was chosen to study the effects of temperature on the performance of the filters. Figure 5.5a–c summarize the removal efficiency for the small solid particles at different temperatures. Particularly for PM$_{0.3}$, one can see from Figure 5.5a that the un-crosslinked sample possesses a slightly higher removal efficiency (99.12 – 99.05 %) within the testing temperature range than that of the crosslinked filter (98.98 – 98.89 %). However, Figure 5.5b and c show that the crosslinked and un-crosslinked filter samples do not exhibit a noticeable dependent behavior on temperature and the removal efficiency for PM$_{0.5}$ and PM$_{2.5}$ is above 99% under all the testing temperatures. In general, it was found that both types of samples possess a very stable performance in the temperature range studied.

In addition to the particulate removal efficiency, the effects of temperature on the toxic chemical removal efficiency of the filters were studied. Figure 5.5d shows the removal efficiency of the crosslinked and un-crosslinked gelatin-based filter mats for HCHO gaseous pollutant. It can be seen that HCHO removal efficiency dropped from 73.42 % to 71.68 % for the un-crosslinked filter
when the temperature increases from 25°C to 45°C, while the crosslinked filter was not noticeably impacted by the temperature change and the removal efficiency rests between 70.97 % and 71.23%. Similarly for the CO gas filtration, we observed a minor drop in filtration efficiency for both types of samples with the temperature increases from 25°C to 45°C and the results are shown in Figure 5.5e. It can be seen that CO removal efficiency decreased from 78.55% to 76.42% and 81.88% to 79.92% for the crosslinked and un-crosslinked samples, respectively. This phenomenon can be explained by the fact that the CO is a linear polar molecules, which interacts with the nanofibers via polar-polar interactions, and increasing temperature can increase the kinetic energy of the gas molecules. A high temperature can decrease the interaction time between the gaseous pollutants and the nanofibers, resulting in a slight reduction of filtration efficiency. It is noted that the crosslinked sample exhibits a more stable toxic chemical removal efficiency compared with that of the un-crosslinked sample. These results, along with the particulate filtration performance, suggest that the crosslinked gelatin-based filtering material possesses very good thermal stability and moisture resistance, which enables the filters to work under different environmental conditions.
FIGURE 5.5. Effects of temperature on the filtration performance for the crosslinked gelatin nanofabrics as compared with the un-crosslinked nanofibers. Particulate removal efficiency for (a) PM$_{0.3}$, (b) PM$_{0.5}$, and (c) PM$_{2.5}$. Toxic chemical removal efficiency for (d) formaldehyde, and (e) carbon monoxide.

5.3.5 Air Flow Resistance at Different Humidity Levels and Temperature

It is believed that environmental conditions such as the humidity and temperature can considerably change the pore structures across the thickness direction of the filter, which can considerably influence the air flow inside the filter. Therefore in this section of the chapter, in addition to the filtration performance, air flow resistance, also known as pressure drop ($\Delta P$), for the crosslinked and un-crosslinked filter samples at different humidity levels and elevated temperatures were
investigated at a fixed flow rate (4 L/min) and the results are shown in Figure 5.6. From Figure 5.6a, one can find that there is a very obvious consistency between the air flow resistance and filtration efficiency. In particular for the un-crosslinked sample, the air flow resistance was increased from 168 Pa to 198 Pa when relative humidity increases from 25% to 75%, then largely escalated to 567Pa for the 95% relative humidity, which has been explained above as the result of film formation due to the dissolution of gelatin nanofibers in high level of moisture content. In contrast, the crosslinked sample exhibited a very stable air flow resistance which lays between 170 and 191 Pa, which is consistent with the previous results on the structure stability of crosslinked filters.

Additionally, we have investigated the effects of temperature on the pressure drop of the un-crosslinked and crosslinked gelatin-based filters and the results are shown in Figure 5.6b. It can be seen that the pressure drop slightly increased with temperature for both types of samples. The un-crosslinked sample possesses a pressure drop ranging from 168 to 182 Pa while the crosslinked sample exhibits an airflow resistance between 170 and 180 Pa when the temperature increases from 25 °C to 45 °C. These results show that the temperature change does not have a noticeable impact on the fiber diameter and pore structures, the two most critical parameters influencing the air flow inside the filter; therefore, both types of samples display a stable air flow resistance behavior versus temperature.
FIGURE 5.6. Effects of (a) humidity and (b) temperature on the air flow resistance of the crosslinked gelatin nanofilter as compared with the un-crosslinked gelatin nanofilter.

5.3.6 Antimicrobial Properties

It is known that bioaerosols and biological contaminants are among the most hazardous pollutants in air, threatening the human health. Moreover, the “green” air filters are made of natural biomaterials, protein nanofibers and a cellulose fiber mat as substrate. Therefore, there is a concern that the protein-based nano-filter may be destroyed by bacteria. Therefore in this section of the chapter, we investigated how the biological pollutants will interact with the crosslinked gelatin-based filter mats. To evaluate the biocide activity of the air filters, *S. oneidensis* and *S. aureus* were selected as gram-negative and gram-positive microorganisms and the qualitative antibacterial evaluation was achieved via the Kirby-Bauer disk susceptibility test.\(^{228,234}\) The reason that *S. aureus* was chosen as the testing microorganism is that *Staphylococcus spp* like *S. aureus* are among predominant airborne bacteria. Moreover, *S. aureus* is known to be the cause of food
poisonings, skin infections and invasive diseases. On the other hand, since *S. oneidensis* is a gram-negative bacterium, in order to test the biocidal effect of the filter materials on gram-positive microorganisms, *S. aureus* was utilized as another testing microorganism. Gram-negative and gram-positive bacteria have structural difference in their cell wall composition. The gram-negative bacteria have a lipopolysaccharides layer at the exterior, which is on top of a thin layer of peptidoglycan. Lipopolysaccharides are composed of covalently linked lipids and polysaccharides with negative charges on lipopolysaccharides. On the other hand, the cell wall of a gram-positive bacterium is composed of a thick layer (∼20–80 nm) of peptidoglycan which consists of linear polysaccharide chains crosslinked by short peptides. Therefore, both gram-negative and gram-positive bacteria were tested in order to see if the sample have biocidal effect against different types of bacteria.

The crosslinked gelatin-based air filter mats and identically sized cellulose paper towel as the control sample were placed on a bacteria inoculated agar plate and were visualized for the biocidal activity after being incubated for 24 hours for the bacteria to grow. The results are shown in Figure 5.7. It was observed that the cellulose paper towel (the substrate for the gelatin/PT filter) did not show any inhibition zone around (see Figure 5.7a and c). This implies that the paper towel itself doesn’t show antibacterial properties. In contrast, when the crosslinked gelatin nanofiber mats were put on the top of the bacteria, a distinctive inhibition zone with size around 3-4 mm wide for *S. oneidensis* and around 1-2 mm wide for *S. aureus* can be clearly observed as shown in Figure 5.7b and d. The inhibition zone indicates that the bacteria around the samples were killed. In other words, the crosslinked gelatin nanofabrics did show interesting antibacterial properties against both gram-negative and gram-positive bacteria. We believe that the antibacterial properties
may be due to the presence of particular functional groups in the structure of gelatin molecules; in particular, some positively charged groups, which can react with the phospholipids and proteins in the cell wall of the bacteria. This mechanism is similar to the antimicrobial mechanism for other antimicrobial agents such as silver and titania nanoparticles. More significantly, the diameter of the inhibition zone did not change after a week in ambient environment indicating an effective and durable biocidal activity for the crosslinked gelatin-based air filtering material. These results demonstrated that, besides the multifunctional air filtration performance, the crosslinked protein-based air filters also possess important antimicrobial properties without incorporation of additional antimicrobial agents such as silver nanoparticles.
FIGURE 5.7. Antimicrobial inhibition zone test against *S. oneidensis* bacteria for (a) cellulose paper towel (PT) as the control sample and, (b) crosslinked gelatin/PT hybrid filter, and against *S. aureus* bacteria for (c) cellulose PT and (d) crosslinked gelatin/PT hybrid filter.

5.4 CONCLUSIONS

In summary, in this chapter, we have successfully demonstrated a type of crosslinked protein nanofabrics for multifunctional air filtration at varying environmental conditions. It is found that the crosslinking for the protein nanofibers can dramatically improve the environmental stability with moisture and temperature, without sacrificing much the filtration performance for both particulate and chemicals pollutants as compared with the one with no crosslinking. Moreover, the crosslinked protein nanofabric also shows antimicrobial properties against the *Shewanella oneidensis* (a gram-negative) and *Staphylococcus aureus* (a gram-positive). The studies in this
chapter indicate that the crosslinked protein nanofabric is a very promising bio-based, multifunctional, and highly efficient air filtering material with antimicrobial functionality and good structure stability for practical applications.
CHAPTER SIX: CONCLUSIONS

For the next generation air filtration systems, the air filtering material must play a multitude of roles: high performance particulate filtration, high efficiency toxic chemical removal, antimicrobial, and low air resistance. These roles determine that an ideal air filtering material accordingly should possess multiple properties simultaneously. The existing air filtering materials have failed in multifunctional air filtering performance in addition to causing tremendous environmental damages due to their non-disposable nature. Meanwhile, recent studies on air filtering materials also simply focused on improving only one air filtering function, either particulate, chemical or antimicrobial. Therefore, development of multifunctional air filtering materials with environmental friendliness is critically needed.

In this dissertation, we studied “green” protein-based multifunctional air filtering materials. We utilize two abundant natural proteins, gelatin and soy protein, to develop high-performance protein-based nanofabric air filter mats. First, uniform gelatin nanofiber mats with very small diameters were fabricated by employing a “green” solvent with optimized composition. Gelatin nanofabrics were fabricated by employing aqueous acetic acid (AA) as a “green” solvent. We studied the effects of solvent composition on the processing of the gelatin nanofibers. The results show that by using the mixed solvent with the AA-to-water ratio of 80 : 20, uniform gelatin filter mats with the nanofiber diameter of 70 nm were successfully fabricated. It has been found that the gelatin nanofiber mats with a controlled uniformity, small fiber diameters and areal density of 3.5 g/m² possess extremely high particulate removal efficiencies of more than 99.3% and 99.6% for PM_{0.3} and PM_{2.5}, respectively. More significantly, the gelatin nanofabrics possess excellent efficiency of absorbing toxic chemicals such as HCHO and CO with efficiencies more than 80% and 76%. These results indicate that the gelatin nanofibers with a much lower areal density of
about 3.43 g/m² can efficiently remove a broad range of PM particles and toxic chemicals compared to one of the most efficient particulate air filters, HEPA with an areal density of 164 g/m². Second, soy protein was employed in a composite as another example to develop sustainable and environmentally friendly nanomaterials for air filtering applications. The soy protein was first denatured using the mixed solvent with the AA-to-water ratio of 80 : 20 to unfold the built-in protein structures. It was found that denaturation can result in a significant reduction in particle size from about 50 μm to less than 30 nm. Then PVA polymer was mixed with denatured soy protein to produce the nanofibers. We studied the role of SPI : PVA ratio on the morphology and performance of the protein-based nanofabric filters. It was found that the SPI/PVA with 1:1 ratio gave rise to uniform nanofiber mats with the diameter of around 130 nm. The soy protein-based nanofabrics with the areal density of 4.50 g/m² showed high removal efficiencies for both types of pollutants, namely, particles with a broad diameter size range and toxic gases with various characteristics while maintaining a low air flow resistance. It was found that the amino acids of proteins can significantly enhance the interactions between nanofabrics and pollutants and the interaction-based filtration mechanism besides the existing size-based primary mechanisms result in the multifunctional filtration performance of the protein-based nanofabrics. The tremendous performance in removal of particulate matter and toxic chemicals simultaneously represents a great potential for advanced air filtration systems.

Further, we have demonstrated that the common paper towel can be engineered with protein nanofabrics to develop a complete disposable air filter with very high filtration efficiencies. The porous paper towels (PTs) are used as substrates, on which the protein nanofibers with 2 g/m² areal density are directly deposited by electrospinning. Firstly, the porous structures and filtration performance of the paper towel substrate were investigated first. The textured cellulose paper
towel was used to promote the mechanical and functional properties of the protein-based filters and regulate the air flow passing through the filter mat. We investigated the effects of the PT substrate on the particulate filtration properties of the GNAF. In particular, different filter configuration for the combination of gelatin nanofabric (G) and the paper towel (PT) substrate has been studied. Owing to the special gradient porous structures of the layered cellulose/protein hybrid filter and the presence of multiple functional groups in the protein structure, the resultant filter is capable of interacting with various pollutants and gives rise to improved removal efficiencies for small particulate pollutants, as compared with pure protein nanofiber filter. Among all types of configuration, the GNAF with G/PT/G configuration shows the highest removal efficiency of 99.30% for the smallest particulate pollutant of PM$_{0.3}$ while maintaining the pressure drop of 195 Pa. It also possesses about 80% removal efficiency for toxic chemical pollutants including HCHO, CO, SO$_2$ and VOCs. This hybrid air filter is made of environmentally friendly and extremely abundant materials, thus it can be used as a disposable device in practice. This study indicates that the porous structures and mechanical flexibility of the common paper towel can provide significant advantages for fabrication of high performance and disposable air filters.

Moreover, the environmental stability of the protein-based nanofabrics is enhanced via crosslinking using a “green” crosslinking agent. Crosslinked protein nanofabrics can significantly improve the structure stability against different moisture levels and temperatures, without sacrificing much the filtration performance for both particulate and chemicals pollutants as compared with the one with no crosslinking while maintaining a low air flow resistance. We first investigated the effect of the crosslinking agent loading on the morphology of the gelatin nanofabrics. The particulate and chemical air filtration performance and the pressure drop of these samples were then tested. It was found that the diameter of the spun fibers increased from 87 nm
to 207 nm after adding the crosslinking agent. Then, the particulate, toxic chemical filtration efficiency and the pressure drop for the samples with different loading of crosslinking agent at 95% humidity were investigated. It was found that there is an optimal loading of the crosslinking agent which can effectively improve the moisture resistance property of the nanofibers and, in the meantime, maintain the mechanical flexibility and the air filtration performance of the filters. To minimize the crosslinking loading, 10 wt% was chosen as an optimized loading of the crosslinking agent for the further testing. We further studied the air filtration performance of the crosslinked air filter mats at different humidity levels and temperatures. The study results indicated that the crosslinked filters possess a very stable filtration properties at different humidity and temperature conditions. Additionally, the antimicrobial properties of the crosslinked hybrid air filters were studied. It was demonstrated that the crosslinked protein-nanomaterials also possess antibacterial properties against the selected gram-negative and gram-positive bacteria. The work presented in this dissertation can greatly facilitate the development of the environmentally friendly, high performance and multifunctional air filters with antimicrobial properties and provide a promising solution for next generation air filtration systems for practical applications without any excessive costs.
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APPENDIX
APPENDIX I

**Gelatin Solution and Nanofiber Mat Preparation.** Gelatin was dissolved in mixed solvent (volume ratio, acetic acid : DI water = 80 : 20) with a concentration of 18 wt% at 65 °C. The mixed solvent was used to achieve a good electrospinning of the gelatin solution. With that ratio between water and acetic acid, it was found that a homogenous yellow solution and stable electrospinning of the solution can be achieved. The electrospinning of gelatin solution is a well-known process and the effect of viscosity on electrospinning of gelatin has been studied before. After the homogenous gelatin solution (18 wt%) is prepared, the nanofibers were spun at room temperature on an aluminum mesh substrate. The areal density of the nanofabrics was controlled by controlling the volume of the solution that was electrospun on the substrate. The gelatin nanofabrics possessed a thickness within the range of 8 – 20 μm. In particular, the sample with the best air filtration properties (areal density = 3.43 g/m²) possesses a thickness of about 16 μm. The gelatin nanofabrics can be handled manually even though they are not crosslinked, but it is challenging due to very high electrostatic charge of the nanofibers. Therefore, the nanofabrics fixed on the aluminum mesh were used for filtration testing. Presence of the aluminum mesh did not affect the filtration properties of the nanofabrics (removal efficiencies and pressure drop) due to very large pore size of the mesh (1 mm x 1 mm).
FIGURE S1.1 Digital picture of the air filtration experimental setup

The digital image of the experimental setup is shown in Figure S1.1. For the air filtration testing, medium and large plastic vacuum air bags (commercial magicbag™) and modified to be able to connect to a home-made filter holder. Each sample was exposed to the air filtration testing for 30 minutes at standard face velocity. Due to the volume limitation of the bags, continuous testing was not possible. Therefore, for long-term filtration testing, we performed multiple numbers of testing with 30 minutes filtration time on each sample.
FIGURE S1.2. Pore size distribution of gelatin nanofabrics.

Pore size is one of the most important parameters affecting the filtration performance as the filtration of particulate pollutants are generally governed by four primary and size-based physical mechanisms, sieving, inertial impaction, interception, and diffusion. This result shows that the gelatin filter nanofabric mats possess the average pore size of around 4.2 μm.

TABLE S1.1. Number concentration of particulate pollutants before and after filtration using gelatin nanofiber filter with areal density of 3.43 g/m².

<table>
<thead>
<tr>
<th></th>
<th>0.3</th>
<th>0.5</th>
<th>1</th>
<th>2.5</th>
<th>5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number [con.] in polluted air</td>
<td>2046623</td>
<td>1753416</td>
<td>962801</td>
<td>218498</td>
<td>15641</td>
<td>12633</td>
</tr>
<tr>
<td>Number [con.] after filtration</td>
<td>13763</td>
<td>14621</td>
<td>1982</td>
<td>314</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>
FIGURE S1.3. SEM images of gelatin filter nanofabrics with different magnifications (a–c) before filtration test and (d–f) after filtration test showing the pollutants were grabbed around the fibers and deformed.

<table>
<thead>
<tr>
<th></th>
<th>Formaldehyde</th>
<th>Carbon monoxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration in polluted air (ppm)</td>
<td>1.75</td>
<td>34</td>
</tr>
<tr>
<td>Concentration after filtration (ppm)</td>
<td>0.3</td>
<td>8</td>
</tr>
</tbody>
</table>

TABLE S1.2. Concentration of toxic gaseous chemicals before and after filtration using gelatin nanofiber filter with areal density of 3.43 g/m².
FIGURE S1.4. SEM images of gelatin air filter nanofabrics after being tested with cigarette smoke showing the deformation and migration of soft PM during filtration procedure, a) first stage, b) semi-saturated stage, and c) saturated stage.
Thermogravimetric analysis (TGA) is utilized to study the thermal stability and degradation of the gelatin nanofibers before and after filtration testing. The test was performed at 20°C/min heating rate. The TGA results show that the gelatin nanofiber mats were very stable in a broad range of temperatures (20–250 °C) both before and after filtration. The filter mats were degraded at around 300 °C.

FIGURE S1.5. Thermogravimetry analysis (TGA) results for gelatin nanofabrics before and after filtration.
The dielectric measurements demonstrated that the commercial HEPA filter, which has no active functional groups in its structure, showed a constant dielectric constant which means, as expected, it is an isolating material. In contrary, gelatin nanofabrics showed higher dielectric values at lower frequencies which means that the gelatin fibers do not have an isolating behavior. In addition, fluctuations in the permittivity values of a material at low frequencies are mostly representative of the rotation and the respond of active functional groups with the electric field to some extent.\textsuperscript{239–242} These results showed a huge amount of fluctuations at lower frequencies for gelatin nanofibers while there was not any changes for commercial HEPA filter that is made of an isolating material. Dielectric test results, as well as FTIR results that have been mentioned in the main paper, proved the existence of many active functional sites to interact with particles and chemicals present in polluted air that resulted in high removal efficiency for both PM\textsubscript{2.5} and toxic chemicals (HCHO and CO) with very low areal density compared with commercial HEPA filter. The quantitative
analysis of air flow resistance was carried out by investigating the pressure drop of the gelatin filter.

The FTIR spectra of gelatin nanofiber before and after the “regular clean air” passes through the filter were collected. It is known that the regular air contains some moisture and particulate pollutants which can be captured by the gelatin nanofabrics. Therefore, it is observed that the intensity of the peak for –OH group was increased slightly which is the result of moisture absorption from the air. However, the intensity of the peaks for other functional groups does not change significantly and no new peak was found for the sample after filtration of regular air.

FIGURE S1.7. FTIR characterization of gelatin filter before and after filtration of regular “clean” air, showing the active functional groups.
APPENDIX II

**Powder-Based Method for Sample Preparation:** Regarding the powder-based procedure, PVA powder was dissolved in 80 % (v/v) aqueous acetic acid at 60 °C for 2hr under magnetic stirring conditions (400rpm). After the PVA had been fully dissolved in the solvent, SPI powder was added in various loadings to the PVA solution and subjected to the same magnetic stirring conditions at 85 °C for 24hr in order to denature SPI in presence of the dissolved PVA. The powder-based procedure samples displayed significantly decreased electrospinability. There was also an issue of the amount of SPI loading permissible in the powder-based samples where above a 1:1 for SPI to PVA ratio, the powder-based procedure failed to produce samples that would survive during the performance testing due to the brittleness of the fiber mat. Considering the disadvantages of power-based method, we finally choose a solution-based or denatured-based method as described in the experimental part in chapter three.
FIGURE S2.1. Particulate removal efficiency of PM$_{2.5}$ for SPI/PVA air filter nanofabrics with different SPI content fabricated via solution-based/denatured-based and powder-based methods.

From this figure, it can be found that solution-based method shows much higher removal efficiency than that of powder-based method.
FIGURE S2.2. Toxic Chemical removal efficiency of a) formaldehyde and b) carbon monoxide for SPI/PVA air filter nanofabrics with different SPI content fabricated via solution-based/denatured-based and powder-based methods. Similar to particulate removal efficiency, solution-based method shows much higher removal efficiency for toxic gases than that of powder-based method.
FIGURE S2.3. Pore size and distribution of SPI/PVA nanofabrics with SPI to PVA ratio of a) 0.6:1, b) 0.8:1, c) 1:1, along with that of d) neat PVA nanofabrics, and e) commercial HEPA filter, f) Illustration of the way to determine the pore size (pore size = \( (d_1 + d_2 + d_3)/3 \)).
FIGURE S2.4. Distribution of the fiber diameter for SPI/PVA nanofabrics with SPI to PVA ratio of a) 0.6:1, b) 0.8:1, c) 1:1, along with that of d) neat PVA nanofabrics, and e) commercial HEPA filter.

FIGURE S2.5. High magnification of the SPI/PVA nanofiber with SPI/PVA ratio of 1:1. a) SEM and b) and c) TEM images. From these images, one can find that there is no SPI nanoparticles formed in the nanofiber, indicating good miscibility between denatured SPI and PVA.
FIGURE S2.6. SEM images of the nanofabrics before air filtration testing: a), pure PVA nanofabric, b) SPI/PVA with ratio of 0.8:1, c) SPI/PVA with ratio of 1.5:1; SEM images of the nanofabrics after air filtration testing: d), pure PVA nanofabric, e) SPI/PVA with ratio of 0.8:1, f) SPI/PVA with ratio of 1.5:1.
FIGURE S2.7. Digital photos of SPI/PVA nanofabrics with high SPI:PVA ratio of 1.5:1. a) Before filtration testing and b) after filtration testing. There are cracks formed after filtration testing, indicating that a high loading of SPI will deteriorate the mechanical strength and finally the filtration performance.
FIGURE S3.1. Digital images of gelatin nanofabric layer: (a) before folding, (b) folded, and (c) after unfolding.
FIGURE S3.2. (a) Tensile test for PT and Gelatin nanofabrics. Digital images at breaking point of (b) PT and (c) gelatin nanofabrics.
TABLE S3.1. Physical properties of different paper towels

<table>
<thead>
<tr>
<th>Properties/ Samples</th>
<th>Scott® PT-T</th>
<th>Scott® PT-P</th>
<th>Bounty® PT-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness (μm)</td>
<td>108</td>
<td>81</td>
<td>183</td>
</tr>
<tr>
<td>Fiber Areal Density (g/m²)</td>
<td>53</td>
<td>50</td>
<td>68</td>
</tr>
<tr>
<td>Avg. Fiber Diameter (μm)</td>
<td>10.4</td>
<td>11.67</td>
<td>12.79</td>
</tr>
<tr>
<td>Avg. Pore Size (μm)</td>
<td>58</td>
<td>52</td>
<td>22</td>
</tr>
<tr>
<td>Surface Texture</td>
<td>Textured</td>
<td>Plain</td>
<td>Highly Textured</td>
</tr>
</tbody>
</table>

FIGURE S3.3. Distribution of the fiber diameter for three types of paper towels: a) Scott® PT-T, b) Scott® PT-P, c) Bounty® PT-B.
FIGURE S3.4. Pore size and distribution of three types of cellulose-based paper towels: a) Scott® PT-T, b) Scott® PT-P, c) Bounty® PT-B.
FIGURE S3.5. SEM images of the surface morphology for the pure gelatin and G/PT nano-filters before and after the filtration testing. The back surface and front surface were characterized as indicated in the figure.
FIGURE S3.6. Fiber diameter and pore size distribution for the protein nanofabrics: a) and b), the pure gelatin nanofabric, c) and d) the protein nanofabric on the PT substrate.
FIGURE S4.1. Microstructures studied by SEM images for the gelatin nanofabrics with different loading of the crosslinking agent: (a) 0%, (b) 5%, (c) 10%, (d) 15%, (e) 20%, and (f) 30%. The scale bar is 5 μm.
FIGURE S4.2. Pore size and distribution of three types of gelatin nanofabrics with different loading of the crosslinking agent: (a) 0%, (b) 5%, (c) 10%, (d) 15%, (e) 20%. 
FIGURE S4.3. Microstructures studied by SEM images for the un-crosslinked gelatin nanofabrics at: a) 25%, b) 60%, c) 75%, and d) 95% humidity levels; and crosslinked gelatin nanofabrics at: a) 25%, b) 60%, c) 75%, and d) 95% humidity levels. The scale bar is 5 μm. The inserts are higher magnification SEM images and the scale bar is 1 μm.