ABSTRACT

PARK, YAEWON. Bioinspired Mineralization of Nanofibers for Water Purification. (Under the direction of Dr. Ericka Ford)

The synthetic modification of textile surfaces using inorganic nanomaterials is crucial to the development of functional textiles, having desirable properties such as water repellency, antimicrobial and water filtration. Nature provides unique examples of nanostructures that are inherently multi-functional. Mimicking these naturally occurring nanostructures will enable the development of novel textile nanotechnologies. In this work, three approaches for inorganic mineralization were studied and all were inspired by nature.

Nanofibrous adsorbents were developed in mimicry of human bone formation. Human bone comprises collagen fibrils and hydroxyapatite nanocrystals, which attach alongside the collagen fibrils. Proteins absorbed into the voids of collagen fibrils nucleate the growth of nanocrystals. Polymerized micelles were synthesized to emulate the macromolecular structure and charge density of proteins. Spherical calcium carbonate (CaCO\(_3\)) particles threaded onto nanofibers upon dipping them into alternating solutions of aqueous calcium chloride (CaCl\(_2\)) and sodium carbonate (Na\(_2\)CO\(_3\)). Polymerized micelles, at the nanofiber surface, attracted Ca\(^{2+}\) and CO\(_{3}^{2-}\) ions to form denser CaCO\(_3\) coatings with the vaterite crystalline form, than other polyelectrolyte seeds. The vaterite phase enhanced anthraquinone dye adsorption from water.

Nanowrinkles formed on the surface of crosslinked poly(vinyl alcohol) nanofibers after repetitive cycles of titanium dioxide (TiO\(_2\)) sol-gel synthesis. This synthetic approach to nanowrinkling mimicked the natural occurrence of skin wrinkling. Wrinkles form when bilayers experience different amounts of strain between each layer. Nanofibers were dipped
in alternating solutions of TiO$_2$ precursors (titanium tetraisopropoxide (TTIP) in isopropanol (IPA) and water for up to 5 cycles. TTIP hydrolyzed to condense in the form of TiO$_2$ nanoparticles. PVA nanofibers swelled in water and deswelled in TTIP solutions. The repetitive swelling-deswelling, focused internal stress along stiff TiO$_2$ nanoparticles to ultimately induce nanowrinkles along the nanofiber surfaces. This unique hierarchical structure could benefit the design of separation membranes for energy storage and superhydrophobic surfaces.

Lastly, manganese oxidizing soil fungi were grown directly onto nanofibers as biocatalysts for mineralization. Several soil fungi can catalyze manganese oxide (Mn(III/IV)O) mineralization by secreting oxidative enzymes that transforms Mn(II) to Mn(IV). Biogenic manganese oxides are known to remove harmful heavy metal ions from water much better than synthetic minerals due to their imperfect crystalline forms. In this study, *Coniothyrium* sp. and *Coprinellus* sp. soil fungi that were isolated from the superfund site (Lot 86, Farm Unit #1) were incubated in the presence of nanofibers. Upon their attachment to nanofibers containing manganese chloride (MnCl$_2$), *Coniothyrium* sp. catalyzed the conformal deposition of Mn(III/IV)O along hyphae and nanofibers, but *Coprinellus* sp. catalyzed Mn(III/IV)O only along its hyphae. Mn(III/IV)O coated nanofibers (with and without *Coniothyrium* sp.) were more effective against Mn(II) and copper (Cu(II)) removal than for lead (Pb(II)). Thus, nanofibrous scaffolds for inorganic nanomaterials and microorganisms can lead to the development of novel functional materials.

Throughout these studies, the surface chemistry of nanofibers- as dictated by seeding with ions, polyelectrolytes or hydroxyl groups populating the surface- was found to have the greatest influence on inorganic mineral nucleation and growth mechanisms.
Bioinspired Mineralization of Nanofibers for Water Purification

by
Yaewon Park

A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy

Fiber and Polymer Science

Raleigh, North Carolina

2018

APPROVED BY:

________________________________________  ______________________________________
Dr. Ericka Ford  Dr. Alan Tonelli
Chair of Advisory Committee

________________________________________  ______________________________________
Dr. Eunkyung Shim  Dr. Saad Khan

________________________________________
Dr. Terrence Gardner
DEDICATION

This dissertation is dedicated to my parents, Heemyung Park and Seungweon Yoon for their unconditional support throughout my Ph.D. work.
BIOGRAPHY

Yaewon Park was born in Seoul, South Korea. She holds a bachelor’s degree in Clothing and Textiles from Seoul National University. During her undergraduate study, she found textiles have a variety of applications in science and technology—not limited to fashion—and developed a huge passion for technical textiles. She pursued the Master of Science in Clothing and Textiles at Seoul National University under the direction of Dr. Chung Hee Park. She joined the Ph.D. program in Fiber and Polymer Science at North Carolina State University as a provost doctoral recruitment fellow. Her doctoral advisor is Dr. Ericka Ford.
ACKNOWLEDGEMENTS

I would like to express my most sincere appreciation to my advisor, Dr. Ericka Ford for her guidance through the entire research. Her advice and support made this research possible. I am very thankful for her valuable advice on career development which has shaped me to be the researcher that I am. I also appreciate the faculty of the Department of Textile Engineering, Chemistry and Science, especially, Dr. Melissa Pasquinelli and Dr. Jeffry Joines for their kind support. I am always inspired to follow in their footsteps to become a great researcher and teacher.

Many thanks to my committee members, Dr. Eunkyoung Shim, Dr. Terrence Gardner, Dr. Alan Tonelli, and Dr. Saad Khan. Their constructive advice and comments were very helpful towards the completion of my research. Dr. Terrence Gardner and Dr. Shuang Liu were amazing collaborators on our innovative work on fungi-nanofiber composites for water filtration.

This research was sponsored by the Nonwovens Institute (14-177 NC) and Department of Textile Engineering, Chemistry and Science. The fourth chapter of this dissertation was funded in part by the North Carolina Sea Grant (NCSG)-Water Resources Research Institute (WRRI) for graduate research.

I was very fortunate to work with great teammates within the Ford Research Team. Preeti Rawat and I worked together to establish electrospinning and characterization techniques. I appreciate her for her assistance in Fourier Transform infrared (FTIR) spectroscopy. Charles Blackwell and Chunhong Lu also provided laboratory assistance and constructive advice.
I owe special appreciation to my friends and family who have supported me in many ways. I would like to express my most sincere gratitude to Sangchul Roh for his support throughout my doctoral study. Without their support, this research would have never been carried out.
# TABLE OF CONTENTS

LIST OF TABLES ......................................................................................................................... x

LIST OF FIGURES ....................................................................................................................... xi

CHAPTER 1. INTRODUCTION ................................................................................................. 1

1.1. Functional Mineral Coating ......................................................................................... 1

1.1.1. Conventional Methods ............................................................................................ 1

1.1.2. Biomimetic Approach ............................................................................................. 1

1.2. Electrospinning Method for Nanofiber Preparation ................................................. 7

1.2.1. Nanofibrous Textiles .............................................................................................. 7

1.2.2. Electrospinning for Mass Production ...................................................................... 8

1.2.3. Process-Structure-Property Relationship of Electrospun Nanofibers .............. 10

1.3. Nanofiber Adsorbents in Water Treatment .............................................................. 11

1.3.1. Adsorption ............................................................................................................. 11

1.3.2. Adsorbent Nanofibers ........................................................................................... 13

1.4. Research Objectives .................................................................................................... 14

CHAPTER 2. BIOMIMETIC CALCIUM CARBONATE COATING OF
NANOFIBERS USING POLYMERIZED MICELLES ......................................................... 16

2.1. Abstract .......................................................................................................................... 16

2.2. Introduction ................................................................................................................... 17

2.3. Materials and Methods .............................................................................................. 20

2.3.1. Materials ............................................................................................................... 20

2.3.2. Methods .................................................................................................................. 21

2.3.2.1. Micellar Particle Formation & Polymerization .............................................. 21
2.3.2.2. Electrospinning Solution ................................................................. 25
2.3.2.3. Electrospinning ............................................................................... 25
2.3.2.4. Crosslinking Nanofibers ................................................................. 26
2.3.2.5. CaCO$_3$ Mineralization of Nanofibers ............................................ 26
2.3.2.6. Nanofiber Characterization ............................................................. 26

2.4. Results and Discussion ......................................................................... 27
  2.4.1. Seeding on Nanofiber Morphology .................................................... 27
  2.4.2. Mineralization by Alternative Dipping Versus Immersion .......... 33
  2.4.3. Effects of Polymerized Micelles on Mineralization .................... 35
  2.4.4. Mineralized Nanofibers as Dye Adsorbents .................................... 37

2.5. Conclusions ........................................................................................ 39

CHAPTER 3. TITANIUM OXIDE SOL-GEL INDUCED NANOWRINKLING OF
ELECTROSPUN FIBERS ............................................................................. 41

3.1. Abstract .................................................................................................. 41
3.2. Introduction ............................................................................................ 42
3.3. Materials and Methods ......................................................................... 44
  3.3.1. Materials ............................................................................................ 44
  3.3.2. Methods .............................................................................................. 45
     3.3.2.1. Electrospinning Nanofibers .......................................................... 45
     3.3.2.2. Crosslinking Nanofibers ............................................................... 45
     3.3.2.3. TiO$_2$ Sol-Gel Synthesis ............................................................... 46
     3.3.2.4. Image Analysis ............................................................................ 47
     3.3.2.5. Spectroscopic Analysis ................................................................. 48
3.4. Results and Discussion ........................................................................................................... 48

3.4.1. 300 mM GA Crosslinked Fiber Morphology After TiO₂ Synthesis .................. 48

3.4.2. 400 mM GA Crosslinked Fiber Morphology After TiO₂ Synthesis .......... 51

3.4.3. Characterization of Wrinkling ........................................... 53

3.4.4. Role of TiO₂ Synthesis on Wrinkling ........................................... 55

3.4.5. Spectroscopy of Nanofiber Surface Chemistry ........................................... 58

3.4.6. Swelling .................................................. 62

2.5. Conclusions......................................................................................................................... 65

CHAPTER 4. MYCOGENIC MANGANESE OXIDE NANOFIBERS FOR HEAVY METAL IONS REMOVAL .................................................................................................................. 66

4.1. Abstract ............................................................................................................................... 66

4.2. Introduction ........................................................................................................................ 67

4.3. Materials and Methods ....................................................................................................... 70

4.3.1. Materials ......................................................................................................................... 70

4.3.2. Methods .......................................................................................................................... 71

4.3.2.1. Nanofiber Preparation ............................................................................................... 71

4.3.2.2. Crosslinking Nanofibers ......................................................................................... 71

4.3.2.3. Media Preparation ..................................................................................................... 72

4.3.2.4. Fungal Treatment of Nanofibers ............................................................................. 72

4.3.2.5. Microscopic Analysis ............................................................................................... 73

4.3.2.6. Spectroscopic Analysis of Mn(III/IV)O ............................................................... 73

4.3.2.7. Heavy Metal Adsorption ......................................................................................... 74

4.4. Results and Discussion ....................................................................................................... 74
4.4.1. Fungal Growth and Mn(III/IV)O Deposition ........................................74
4.4.2. Elemental Analysis of Fungal Mn(III/IV)O Hybrids ..................................79
4.4.3. Mn(III/IV)O Deposition on Nanofibers .....................................................81
4.4.4. Heavy Metal Adsorption by Coniothyrium sp. Hybrids ............................86

4.5. Conclusions ......................................................................................................89

CHAPTER 5. SUMMARY & CONCLUSIONS .............................................................90

APPENDICES .........................................................................................................93

APPENDIX A. Properties of Surfmer Solutions ......................................................94
APPENDIX B. Fiber Properties After TiO2 Induced Wrinkling ..............................95
APPENDIX C. Morphology of Manganese Oxidizing Fungi-Nanofiber Hybrids ......99

REFERENCES .......................................................................................................101
LIST OF TABLES

Table 1.1. Fiber Production Efficiency of Different Electrospinning Set-ups for PVA Nanofibers .................................................................10

Table 2.1. Effect of Seeding on the Solution Conductivity and Size of Nanofibers from Aqueous PVA Solutions.................................................28

Table 2.2. Dye Adsorbed by Mineralized Nanofibers Containing Different Seeds from 10 mg/L Acid Blue Dye Solutions. Untreated PVA indicates crosslinked PVA nanofibers before mineralization.......................................................39

Table 3.1. Crystallinity and Acetal Bridge Formation of PVA Fibers Indicated by FTIR Spectra..............................................................................60

Table 3.2. Swelling of Electrospun PVA Nanofibers after 24 Hours of Water and IPA Immersion.............................................................................63

Table 4.1. Summary of Nanofibers with and without Fungal Treatment ........................................73

Table 4.2. Comparing Aqueous Heavy Metal Adsorption by Coniothyrium sp. Derived Mn(III/IV)O Coatings and Nanofibers After 24 h ........................................87
LIST OF FIGURES

Figure 1.1. Importance of proteins in biomineralization of (a) human bone and (b) nacre.
   Schematic shows that (i) highly organized nanocrystals are formed along the scaffolds proteins and (ii) small proteins with functional groups nucleate and guide crystal growth in both bone and nacre.................................................................3

Figure 2.1. Graphical abstract of Chapter 2 ..................................................................................16

Figure 2.2. Chemical structures of (a) PAMS (b) PAPS, and (c) HEA. The schematic represents surfmer-micelles before and after polymerization in (d). Reacted micelles contained PAPS or PAMS with comonomer HEA, whereas unreacted micelles did not contain HEA ........................................................................................................22

Figure 2.3. 1H NMR spectra of unreacted and reacted (a) PAMS and (b) PAPS. .....................23

Figure 2.4. The particle size analysis of 0.1 wt% surfmer in water is shown for micelles of unreacted and reacted PAMS and PAPS. Solutions were also diluted by 50% ...25

Figure 2.5. The electrospun morphologies of untreated (a) PVA, (b) CaCl₂/PVA, (c) PAMS/PVA, (d) reacted PAMS/PVA, (e) PAPS/PVA, and (f) reacted PAPS/PVA nanofibers are shown .................................................................29

Figure 2.6. XPS C 1s spectra of untreated (a) PVA, (b) CaCl₂/PVA, (c) PAPS/PVA and (d) reacted PAPS/PVA nanofibers along with deconvoluted peaks are shown ..........31

Figure 2.7. High resolution scans of XPS spectra for (a) Ca 2p and (b) Cl 2p in CaCl₂/PVA nanofibers, (c) S 2p in PAPS/PVA and (d) S 2p in reacted PAPS/PVA..........32

Figure 2.8. The surface chemistry of seeded nanofibers is illustrated, as evidenced by XPS ..................................................................................................................................................33
Figure 2.9. Morphologies of CaCO$_3$ mineralized PAMS/PVA nanofibers by alternating dip cycles of aqueous CaCl$_2$ and Na$_2$CO$_3$ are shown for (a) 10, (b) 15, and (c) 20 cycles. Nanofibers were dipped into CaCl$_2$/Na$_2$CO$_3$ solution for 1 week (d). CaCO$_3$ mineralized CaCl$_2$/PVA nanofibers by alternating dip cycles for (e) 10, (f) 15, and (g) 20 cycles are shown.

Figure 2.10. Mineralized (a) PVA (b) CaCl$_2$/PVA (c) PAMS/PVA (d) reacted PAMS/PVA (e) PAPS/PVA and (f) reacted PAPS/PVA nanofibers are shown. These fibers were mineralized by alternating dipping cycles ten times.

Figure 2.11. Raman spectra from mineralized nanofibers of PVA with and without seed particles. Untreated PVA indicates crosslinked PVA nanofibers before mineralization.

Figure 2.12. Photograph of Acid Blue 25 adsorbed by mineralized (a) CaCl$_2$/PVA nanofibers revealed the checkered pattern of nanofibers electrospun onto aluminum mesh. The structure of the (b) anthraquinone dye is shown. The contrast in dye absorbed from 10 mg/L solutions is shown for different samples of mineralized nanofibers (c).

Figure 3.1. Graphical abstract of Chapter 3.

Figure 3.2. Photographs show electrospinning on the a) lab-scale to produce 15 kV nanofibers and b) pilot scale to produce 45 kV nanofibers.

Figure 3.3. Micrographs of PVA nanofibers electrospun at (a) 15 and (b) 45 kV are shown (i) before and (ii) after crosslinking with 300 mM GA. Crosslinked nanofibers were dipped in alternating solutions of TTIP and water for (iii) 1 (iv) 3 and (v) 5 cycles.
**Figure 3.4.** Micrographs of PVA nanofibers electrospun at (a) 15 and (b) 45 kV and crosslinked with 400 mM GA are shown (i). Crosslinked nanofibers were dipped in alternating solutions of TTIP and water for (ii) 1 (iii) 3 and (iv) 5 cycles. 52

**Figure 3.5.** AFM phase and height images of 45 kV, 300 mM GA nanofibers that were treated with (a) 0 and (b) 5 cycles of TiO₂ sol-gel immersion, where (c) shows the roughness of TiO₂ coatings along the line shown in (b) that crosses a nanofiber. 55

**Figure 3.6.** Raman spectra of 15 kV-300 mM GA and 45 kV-300 mM GA nanofibers treated with up to 5 cycles of TiO₂ sol-gel coating. 57

**Figure 3.7.** FTIR spectra of PVA nanofibers that were electrospun at 15 or 45 kV before crosslinking with different GA concentrations. 58

**Figure 3.8.** XPS C 1s peaks of electrospun nanofibers designated as (a) 15 kV, 300 mM GA, (b) 45 kV, 300 mM GA, (c) 15 kV, 400 mM GA, and (d) 45 kV, 400 mM GA. 61

**Figure 4.1.** Graphical abstract of Chapter 4. 66

**Figure 4.2.** The schematic shows the laboratory-scale electrospinning set-up. 71

**Figure 4.3.** Fungi (a) *Coprinellus* sp. and (b) *Coniothyrium* sp. were grown for 1 week in Leptothrix media with Mn(II) or without Mn(II) (i.e. -Mn(II)). Fungi (a and b) were incubated without nanofibers (of 1x1 cm²) or with nanofibers. Nanofibers were either of neat PVA or PVA/MnCl₂. SEM images of (c) PVA and (d) MnCl₂/PVA nanofibers are shown. Photographs show (e) *Coniothyrium* sp. grown for up to 4 weeks in Mn(II)-containing media and PVA nanofibers or
PVA/MnCl$_2$ nanofibers of 4x5 cm$^2$. This growth pattern was also observed for Coprinellus sp.................................................................76

**Figure 4.4.** Micrographs show (a) Coprinellus sp. (b) Coniothyrium sp. grown on PVA and PVA/MnCl$_2$ nanofibers immersed in -Mn(II) or +Mn(II) liquid media............78

**Figure 4.5.** EDS graphs of Coprinellus sp. (a) Coniothyrium sp. (b) on PVA (i, iii) or PVA/MnCl$_2$ nanofibers (ii, iv) in liquid media -Mn(II) and with +Mn(II) .............80

**Figure 4.6.** SEM images of Mn(III/IV)O$_2$-#17-PVA/MnCl$_2$ after water immersion and magnetic stirring for 30 min at 500 rpm; both (a) longitudinal and (b) cross-sectional views are shown. Hybrids were further treated with (c) 1 h of sonication in water or (d) 2 h of sonication in ethanol..........................................................82

**Figure 4.7.** (i) SEM images and (ii) EDS spectra from Coprinellus sp. incubated with (a) PVA and (b) PVA/MnCl$_2$ nanofibers are shown. (i) SEM images and (ii) EDS spectra of Coniothyrium sp. incubated with (c) PVA and (d) PVA/MnCl$_2$ nanofibers are shown.................................................................83

**Figure 4.8.** EDS map of Mn(III/IV)O$_2$- PVA/MnCl$_2$ (#17): C in red, Mn in green, and O in blue .................................................................84

**Figure 4.9.** Raman spectra of nanofibers treated with Coprinellus sp. and Coniothyrium sp. ........................................................................................................................................85

**Figure 4.10.** EDS chemical mapping of (a) PVA/MnCl$_2$ and (b) Mn(III/IV)O$_2$- PVA/MnCl$_2$ (#17) nanofibers after Cu(II) adsorption: (i) C in red, (ii) O in blue, (iii) Cu in green, and (iv) Mn in cyan .................................................................88
CHAPTER 1

INTRODUCTION

1.1. Functional Mineral Coating

1.1.1. Conventional Methods

Inorganic coatings are facile techniques\(^1\) for imparting functionality—such as water/oil repellency, anti-static, and antibacterial properties, etc—\(^2,3\) to textiles. Inorganic coatings in the form of nanoparticles have attracted much interest since they allow textiles to maintain important properties such as pore structure, breathability, hand, etc.\(^3\) Also, it is well known that the high surface area per unit mass of nanoparticles enhance textile properties greatly in compared to their micron-sized counterparts.\(^4\)

Metal oxide nanoparticles (MNOP) are especially popular for adding multi-functionality to textiles.\(^1\) MNOPs are prepared by various routes; such as chemical vapor deposition, sol-gel coating, precipitation, and aerogel methods.\(^4\) The sol-gel method is widely used for textiles because it does not require harsh conditions which can degrade textiles. Nanoparticles smaller than 50 nm are prepared by the sol-gel method. Nano-particulates from inorganic sols (i.e. nanosols) are usually prepared by the acid or alkali hydrolysis of metal alkoxides in ethanol. Nanosol coated textiles are then dried to condense nanosol particulates in the form of a gel.\(^1,5\)

1.1.2. Biomimetic Approach

Biomineralization inspires the environmentally friendly and controlled growth of inorganic coatings.\(^6\) Biomineralization describes the highly organized, inorganic synthesis of minerals by living systems; namely bone and nacre formation.\(^7,8\) Bone consists of hydroxyapatite (Ca\(_{10}(PO_4)_{6}(OH)_2\)) nanocrystals attached along the fibrillar axes of collagen
proteins. The hierarchical structure is believed to result in bone’s tough and light-weight properties. The mineralization mechanism of collagen fibrils is not clearly understood; nevertheless, some researchers believe proteins have critical roles in the process of mineralization. Acidic non-collagenous proteins are absorbed into the voids of collagen fibrils to initially nucleate and stabilize amorphous calcium phosphate within the collagen fibrils. The charged surface of proteins seems to control the nucleation and growth of inorganic minerals (Figure 1.1a).

Nacre develops its highly organized shape by a similar mechanism of mineralization to bone although its formation is also largely unknown. Nacre consists of layered aragonite-CaCO$_3$ platelets of ~200-900 nm in thickness and 5-8 µm diameter- that are bound by a thin organic layer of 10-50 nm. Macromolecular proteins are thought to play a critical role in mineralization. One plausible model describes how water insoluble macromolecules such as fibrous collagen, chitin and polysaccharides provide frameworks for crystal growth. Water soluble proteins adsorb onto the water insoluble proteins; this maximizes hydrophobic interactions. Acidic functional groups along the water-soluble proteins align themselves in a controlled fashion at the interface of water insoluble substrates. Metal ions are attracted by surface functional groups and ultimately result in crystal formation (Figure 1.1b).
Figure 1.1. Importance of proteins in biomineralization of (a) human bone and (b) nacre. Schematic shows that (i) highly organized nanocrystals are formed along the scaffolds proteins\textsuperscript{8,9} and (ii) small proteins with functional groups nucleate and guide crystal growth in both bone\textsuperscript{11} and nacre\textsuperscript{12}.

A number of microorganisms such as bacteria, algae and fungi produce inorganic coatings.\textsuperscript{12} For example, manganese (Mn) oxidizing bacteria and fungi\textsuperscript{13,14} form mineral coatings that are able to remove a variety of heavy metals from the environment. Proteins mediate the fungal hydrolysis and synthesis of silica and titania nanoparticles.\textsuperscript{15} Interestingly, biogenic coatings of inorganic nanomaterials can exhibit superior functions to synthetic ones.\textsuperscript{12} Biomineralization has not been as extensively studied as the synthetic hydrothermal route. Nevertheless, nanomaterial synthesis through microorganisms can provide novel and
environmentally friendly techniques for mineralization. The challenge of biomimicry is that mineralization differs largely between microorganisms.

In general, Mn(II) or Mn$^{2+}$ is the most thermodynamically stable form of manganese; however, Mn(II) can oxidize to Mn(III) or Mn(IV) with the help of microorganisms. Bacteria and other microorganisms catalyze Mn(II) oxidation by modifying the local pH of water or by releasing catalytic macromolecules, i.e. polysaccharides or proteins. Santelli et al revealed that different fungi produce manganese oxides having different morphologies. Although reaction conditions were similar, morphologies did depend on whether the reaction site was on the hyphae surface or some distance from fungal hyphae. Plectosphaerella cucumerina and Pyrenochaeta species produced manganese oxides on the surface of fungal hyphae - which is a filamentous structure of fungi -, which is an indication of enzymes or reactive superoxides (O$_2^-$) near the fungal cell wall. Stagonaspora species produced manganese oxides away from the cell wall. Anionic polymers (such as acidic polysaccharides and a Mn(II)-oxidizing protein) secreted from a fungus were believed to template the growth of manganese oxide.

Many studies had coated textiles with inorganic nanomaterials through the biomimetic approach. A variety of factors- including surface chemistry of the macromolecular matrix, transformation from amorphous phase, environmental conditions such as temperature and pH - affect mineralized morphologies and properties. Surface functionality had an important role in mineralization. Lakshminarayanan et al achieved an abundance of functional groups at the surface of commercial fibers through acidic and alkali treatments, which promoted nucleation. Also, different functional groups caused the formation of different CaCO$_3$ polymorphs. Poly(acrylic acid) was shown to nucleate and modify crystal growth at the
surface of electrospun cellulose acetate fiber surfaces; this was attributed to the abundance of carboxylic acid groups.\(^{22}\) Another widely used method is to incorporate biomacromolecules within fibers. Yang et al\(^ {23}\) added chitosan in combination with CaCO\(_3\) nanoparticles and CaCl\(_2\) salts to mineralize electrospun fibers with CaCO\(_3\). The high charge density of chitosan alongside CaCO\(_3\) nanoparticles and CaCl\(_2\) provided nucleation sites for the heterogeneous nucleation of CaCO\(_3\) at the fiber surface. Self-assembled layers of amphiphilic peptides make the surface of nanofibers\(^ {24}\) good templates for hydroxyapatite mineralization. Genetically engineered proteins\(^ {11}\) also make good templating agents. Surfactant micelles serve as nucleating agents for crystal growth, as biomacromolecules.\(^ {25,26}\)

Biogenic mineralization of fibers has not been extensively studied despite its potential use as an environmentally sustainable manufacturing technique. El-Rafie et al\(^ {27}\) treated cotton textiles with silver nanoparticles that were produced by fungi. These particles were used to develop antimicrobial textiles. Therefore, other biogenic approaches for the formation of nanomaterials—such as silica, titania and manganese oxides—are worthwhile for the study of multifunctional textile coatings.

Moreover, depositing stiff inorganic layers on soft polymers allows the creation of hierarchical structures, namely wrinkles. Natural wrinkles are found among bacterial biofilms and along human skin.\(^ {28}\) Bacterial cells are embedded within the extracellular matrix (ECM) of biofilms. Structural proteins, polysaccharides, and carbohydrates comprise the ECM. Biofilm wrinkling is caused by the localization of stiff dead cells that reside among new cells and ECM. Dead cell regions act as focal points of wrinkling.\(^ {29}\) The wrinkling of human skin results from similar processes. Skin consists of two main layers: epidermis and dermis. Epidermis is an outer-layer of mostly dead cells, while the dermis sublayer has living cells.
throughout its ECM.\textsuperscript{30} Wrinkling occurs with aging, as skin’s outer layer stiffens relative to inner layers and as skin repeatedly undergoes muscular tightening and relaxation.\textsuperscript{31} The loss of elastic collagen and elastin fibers from skin’s outer layer are other causes of stiffening. The degree of differences in positional strain affects wrinkling morphology.\textsuperscript{30} Mathematical models are used to predict that waveform of wrinkles along thin films resting on top of a thick soft elastic foundation.\textsuperscript{30,32} The compressive force between the skin is expressed as Equation 1.1\textsuperscript{30}: \[
F = E_s \left( \frac{\pi^2}{\lambda} \frac{w h^3}{3(1-\nu_s^2)} + \frac{\pi}{\lambda} \frac{E_f w}{4(1-\nu_f^2) E_s} \right)
\] where $\lambda$ is the wrinkle wavelength, $h$ is the skin thickness, $\nu_s$ and $\nu_f$ is the Poisson ratio of the skin and the foundation; $E_s$ and $E_f$ is the modulus of the skin (coated layer) and the foundation (polymer). When a critical stress is exceeded, a wrinkle is formed along the direction of applied compressive force and $\lambda$ is governed by $h$ as in Equation 1.2\textsuperscript{30}: \[
\lambda = 2\pi h \left( \frac{(1-\nu_f^2) E_s}{(1-\nu_s^2) E_b} \right)^{1/3}
\] Calculated wavelengths from Equation 1.2 were in good agreement with the measured wrinkle wavelengths in micro\textsuperscript{33} and nanoscale wrinkles\textsuperscript{34,35} on polymer substrates.

Another important mathematical model describes that wrinkle is formed when the sum of potential energy of bending of the skin and the stretching energy of the foundation is minimized as Equations 1.3 and 1.4\textsuperscript{32}: \[
\lambda \sim \left( \frac{B}{f} \right)^{1/4}
\]
\[
A \sim \left( \frac{\Delta}{w} \right)^{1/2} \lambda
\] where $B$ is the bending stiffness of the skin, $T$ is the stiffness of the effective elastic foundation, $A$ is the amplitude of wavelength and $\frac{\Delta}{w}$ is an imposed compressive strain. The
model applies to any geometries, but on flat surfaces Equation 1.3 reduces to \( \lambda \sim \left( \frac{E_s}{E_f} \right)^{1/3} \) in accordance to Equation 1.2. The mathematical models accurately predicted microscale wrinkles on polymer substrates created by pre-strain and solvent diffusion.

1.2. Electrospinning Method for Nanofiber Preparation

1.2.1. Nanofibrous Textiles

Textiles have several advantages over films and porous membranes. The majority of textiles have solid fraction of less than 0.5, which leads to good air and water vapor transport properties. Proper design of pore size, geometry, and tortuosity can give desirable barrier performance. Surface area per a given fabric volume (i.e. specific surface area) was found to govern air filtration. The specific surface area \( (S_v) \) of circular fibers are calculated by Equation 1.5:

\[
S_v = \frac{2\pi rl}{\pi r^2} = \frac{2}{r} \quad (1.5)
\]

where \( r \) is radius of fiber cross-sections and \( l \) is length of fiber. Thus, fiber diameter is inversely related to the specific surface area of fibrous materials. In this sense, nanofibers have outstanding advantages over conventional micron-sized fibers that are associated with large specific surface area and nanoscale porosity. The surface functionalization of nanofibers with specific functional groups or nanoparticle coatings can enhance the capture or filtration properties of textiles. Moreover, nanofibers are arguably the most ideal form for mimicking complex structures as found in nature. Such mimicry will broaden the functionality of textiles and applications for polymer nanocomposites. Among the variety of nanofiber manufacturing methods, electrospinning is most conducive to mass production-thanks to extensive studies on process-structure-property relationships and equipment design.
1.2.2. Electrospinning for Mass Production

Electrospinning is known to be the simplest and the most cost effective way to form nanofibrous webs.\textsuperscript{40} This technique allows the production of fibers, having diameters in the range of 3 nm - 5\textmu m.\textsuperscript{42} Laboratory set-ups for electrospinning typically comprise a syringe pump, high voltage source and collector. As high voltage is applied to the polymer solution, a Taylor cone is formed at the syringe tip. As the electric field overcomes the surface tension of the polymer solution, the charged droplet forms a jet. As the polymer jet further stretches under the electric field, solvent continuously evaporates. Once the polymer jet reaches the collector, it solidifies as a fibrous web.\textsuperscript{41,43}

The commercial application of electrospinning was initially limited by poor production efficiencies.\textsuperscript{44} Since then, multi-jet\textsuperscript{44} or roller spinning set ups\textsuperscript{45,46} were designed to improve productivity. Laboratory scale electrospinning equipment is simply modified with multiple jets to improve efficiency. Several research groups have utilized multi-jet devices to fabricate nanofibrous textiles.\textsuperscript{47–49} Further, multi-jet electrospinning is a facile method for blending different materials- each from a different syringe- in a controlled manner\textsuperscript{48,49}. Electrical fields surrounding each jet will often interfere with other jets. This is a significant drawback to using multi-jet electrospinning.\textsuperscript{47,50} Roller electrospinning devices comprise of a rolling electrode that is partially immersed in polymer solution. Above the roller lies the collector electrode. Multiple Taylor cones for nanofiber spinning are created at the surface of the rotating electrode, as such the technology is highly productive.

Innovenso Ltd. and Elmarco, Inc. are renowned suppliers for industrial scale electrospinning equipment. Innovenso Ltd. provides commercial multi-jet equipment while Elmarco, Inc. manufactures needle-less electrospinning equipment that includes a roller or
stationary wires as electrodes. Both companies claim fiber production rates up to 200 g/h (3.3 g/min), which is almost three times greater than lab scale electrospinning. The industrial-scale manufacturing of nanofibrous textiles by electrospinning was estimated to cost $1-5/kg. This makes electrospun nanofibers a cost-effective platform for drug delivery.\textsuperscript{51}

Electrospinning conditions and production efficiencies for poly(vinyl alcohol) (PVA) produced by different electrospinning set-ups are compared in Table 1.1. Fiber production efficiency, defined by the mass of spun fiber per minute, differed by several orders of magnitudes depending on the type of electrospinning equipment. If PVA fiber production efficiency was noted as flow rate [mL/h] in the reference, it was converted to [g/min] based on the assumption that PVA polymer density is 1.19-1.3 g/cm\textsuperscript{3} \approx 1 g/cm\textsuperscript{3}. As shown in Table 1.1, fiber production by needle-less electrospinning is more efficient than single and multi-jet configurations.
### Table 1.1. Fiber Production Efficiency of Different Electrospinning Set-ups for PVA Nanofibers

<table>
<thead>
<tr>
<th>Electrospinning Set-up</th>
<th>Polymer Concentration (wt%)</th>
<th>Applied Voltage (kV)</th>
<th>Fiber Production Efficiency (g/min)</th>
<th>Fiber Diameter (nm)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab Scale Single Jet</td>
<td>10</td>
<td>20</td>
<td>0.5mL/h ≈ 0.8 x 10⁻³</td>
<td>-</td>
<td>52</td>
</tr>
<tr>
<td>Lab Scale Single Jet</td>
<td>7</td>
<td>19</td>
<td>1 mL/h ≈ 1.6 x 10⁻³</td>
<td>240 - 290</td>
<td>53</td>
</tr>
<tr>
<td>Lab Scale Multi-jet</td>
<td>10</td>
<td>20</td>
<td>7.6 x 10⁻³</td>
<td>190</td>
<td>49</td>
</tr>
<tr>
<td>Pilot Scale</td>
<td>7.8 – 17</td>
<td>60</td>
<td>2.9 x 10⁻² – 1.3 x 10⁻¹</td>
<td>175 - 305</td>
<td>46</td>
</tr>
</tbody>
</table>

1.2.3. Process–Structure–Property Relationship of Electrospun Nanofibers

Various factors influence fiber formation by electrospinning. Those factors include solution properties such as viscosity, conductivity and surface tension; process variables such as voltage and tip to collector distance; and ambient conditions such as humidity and temperature. When polymer concentration is too low, beaded fibers are spun. When polymer concentration is too high, electrospinning becomes difficult or even impossible at high polymer viscosities. Increasing the solution conductivity or charge density of polymer
solutions is accomplished by the addition of salts or surfactants. As a result, more uniform fibers having less beads are formed as whipping instabilities and elongational forces are exerted on the highly conductive jet. Tip to collector distance (TCD) also affects fiber morphology. Too large TCD lessens the ability of the jet to stretch under the applied electric field. If TCD is too small, less stretching occurs and solvent evaporation is reduced— as a result, fibers may stick to each other. As higher voltages are applied, fiber diameter distributions become broader and beaded fibers may form. Applied voltage mostly affects the mass of polymer ejected from the needle, but there are no significant effects on fiber diameter in comparison to other factors. At high humidity, the phase separation of polymer and solvent at the surface of electrospun fiber created porous fibers.

Polymer solutions are charged with an applied positive or negative voltage bias during electrospinning. Core-shell morphologies can develop as molecules within polymer solutions separate under the applied electric field. Researches have reported the location and orientation of surfactants, proteins, and functional polymers can be manipulated by applied voltage during electrospinning. The electric field outside of the Taylor cone is higher than the inside. This causes a high electrical field gradient. Polarizable molecules can migrate towards the surface of the Taylor cone via dielectrophoresis.

1.3. Adsorbent Nanofibers for Water Treatment

1.3.1. Adsorption

Water treatment is crucial to maintain the sustainability of human health, safety, and environmental preservation. Many methods of water purification have been suggested including chemical precipitation reactions, ion exchange, adsorption or bioremediation. Chemical precipitation is most widely used industrially due to the simplicity of reactions and
relatively low cost. Heavy metal cations react with hydroxide or sulfide anions in the form of water insoluble salts. However, toxic by-products can persist upon reaction. Ion exchange is a highly efficient, high capacity technique for heavy metal removal. Heavy metal ions replace protons along the sulfonic acid (-SO$_3$H) or carboxylic acid (-COOH) groups that populate surfaces of polymeric beads or fibrous filter media. Adsorption might be the most recognized water treatment method for various contaminants such as heavy metals and dyes due to its low cost and flexible design and operation. Activated carbon, inorganic nanomaterials, lignin, zeolites, crab shell, microbial biomass including bacteria and fungi have been studied as adsorbents.

Adsorption is generally defined as the increased concentration of a fluid onto the surface of a solid. The solid, having an adsorption site, is referred to as an adsorbent. Adsorbed species are called adsorbate. Changing fluid conditions (such as concentration, temperature, pH) can cause adsorbed substance to detach from the adsorbent surface. This is referred to as desorption. Adsorption can be loosely divided into physical adsorption and chemical adsorption (chemisorption), which involves chemical interactions. Adsorption occurs on the surface; therefore surface area is of critical importance.

Langmuir, Freundlich, and BET (Brunauer-Emmet-Teller) isotherm models were used to describe the equilibrium adsorption of inorganic materials. The Langmuir model assumes monolayer adsorption without any interaction between adsorbates and adsorbents. The linear form of a Langmuir isotherm is described as Equation 1.6:

$$\frac{C_e}{q_e} = \frac{1}{Q_0b} + \frac{C_e}{Q_0} \quad (1.6)$$
where $C_e$ is the equilibrium concentration, $q_e$ is the amount of adsorbate in the adsorbent at equilibrium, $Q_0$ is the maximum monolayer coverage capacities and $b$ is the Langmuir isotherm constant.

The Freundlich isotherm is an empirical model for a heterogeneous surface. It supposes multilayer adsorption with non-uniform affinities of adsorbate for adsorption sites. The linear form of the Freundlich equation is described as Equation 1.7:

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \quad (1.7)$$

where $K_F$, the Freundlich isotherm constant- relates to adsorption capacity and $n$ is adsorption intensity or surface heterogeneity.

The BET isotherm is the most widely used theory of gas-solid equilibrium. It assumes multilayer adsorption without any interaction between subsequent adsorption layers. The linear form of the BET equation for liquid-solid interface is described as Equation 1.8:

$$\frac{C_e}{q_e(C_s-C_e)} = \frac{1}{q_s C_{BET}} + \frac{(C_{BET}^{-1}) C_e}{q_s C_{BET} C_s} \quad (1.8)$$

where $C_s$ is the adsorbate monolayer and saturation concentration, $q_s$ is the theoretical isotherm for saturation capacity, and $C_{BET}$ is the BET adsorption isotherm relating to the energy of surface interaction.\textsuperscript{73}

**1.3.2. Adsorbent Nanofibers**

Several studies report nanocomposite fibers are highly efficient adsorbents since inorganic nanoparticles are fixed onto fiber substrates- thereby preventing the contamination of the fluid phase.\textsuperscript{74} and have high surface area.\textsuperscript{40} Commercial adsorbents such as activated carbon, zeolites, CaCO$_3$, and metal oxides nanoparticles can be attached to nanofiber surfaces to develop new adsorbents.\textsuperscript{75} Electrospun adsorbents were shown to remove heavy metal ions, organic pollutants and microbes from contaminated water.\textsuperscript{40,75}
The adsorption of contaminants is affected by various factors. Heavy metals adsorb by ion-exchange and precipitation onto mineralized surfaces; therefore, high specific surface area is a crucial parameter for decontamination. Several researchers reported surface charge is more important than specific surface area for cationic dye adsorption. Yamanaka et al. observed that the CaCO₃ crystal phase had a significant role in the adsorption of formaldehyde vapor. As the vaterite of the CaCO₃ polymorph fraction increases, rather than the calcite fraction, formaldehyde vapor adsorption was more favorable.

### 1.4. Research Objectives

The inorganic mineralization of nanofibers can lead to the development of multifunctional textiles. As a result of this research, we sought to develop functional coatings by biomimetic approaches. Common to work featured in the following chapters (and literature) is the use of heterogeneous nucleation or catalysis of inorganic minerals to benefit the formation and performance of hybrid/composite materials.

The research objectives of this work were to

1. investigate surface chemistry and macromolecular influences on biomimetic coatings
2. study a biogenic approach to nanofiber mineralization
3. compare and contrast mechanisms of water decontamination by mineralized nanofibers.

In chapter 2, supramolecular structure and high surface charge of biomineralization-promoter protein was emulated with polymerized micelles. In human bone formation, small proteins are adsorbed into the voids within collagen fibrils to nucleate and grow hydroxyapatite crystals along the collagen fibrils. Effects of polymerized micelles embedded
in nanofibers on the degree of CaCO$_3$ coating, CaCO$_3$ morphology and crystalline phase and anthraquinone dye adsorption were investigated.

In chapter 3, nanowrinkles were created along the crosslinked PVA fibers by mimicking natural wrinkling. Wrinkles are formed on human skin or bacterial biofilm due to the differences in strain between layers. Stiff layers on the top of soft foundations focus internal stresses induced by deformation which lead to wrinkling. Fiber surface chemistry (-OH group population), TiO$_2$ coating on nanofiber surfaces and strain induced by swelling-deswelling of the crosslinked PVA fibers were studied and linked to aligned wrinkling along the electrospun fiber axis.

In chapter 4, manganese oxidizing fungi were used to develop novel composites for water filtration. Specific species of fungi exude oxidative enzymes that are capable of oxidizing Mn(II) to Mn(IV). So as to take advantage of oxidative enzymes for manganese oxide formation, microscale filamentous fungi were grown on the surface of nanofibers. Nanofibers were deemed ideal substrates to hold fungal hyphae and biogenic manganese oxides, which are well known natural adsorbents for heavy metals. Effects of seeding nanofibers with manganese chloride (MnCl$_2$) ions and biogenic manganese oxide formation on the nanofiber surfaces by different species of fungi on the heavy metal removal properties were also investigated.
CHAPTER 2

BIOMIMETIC CALCIUM CARBONATE MINERALIZATION OF NANOFIBERS

USING POLYMERIZED MICELLES

2.1. Abstract

Calcium carbonate (CaCO₃) is a well-known natural adsorbent. In this study, anthraquinone dye adsorption by CaCO₃ mineralized nanofibers was evaluated with respect to the chemistry and structure of ionic particles that were seeded into fibers. Reacted and unreacted “surfmers” of polyoxyethylene-1-(alkyloxyethylmethyl) alkyl ether sulfuric ester ammonium salt (PAMS) and polyoxyethylene alkylphenyl ether ammonium sulfate (PAPS) were added to aqueous polyvinyl alcohol (PVA) solutions at concentrations above their critical micelle concentration prior to electrospinning. The roles of these polymerized

---

1 The material in this chapter was published as: Park Y, Rawat P, Ford E. Role of Polymerized Micelles on the Calcium Carbonate Mineralization of Nanofibers. Industrial & Engineering Chemistry Research, 2017, 56, 8241-8250.
micelles on CaCO$_3$ mineralization (produced by dipping PVA nanofibers into alternating solutions of aqueous CaCl$_2$ and NaCO$_3$) were compared to calcium chloride (CaCl$_2$) and unseeded PVA nanofibers. Seeding nanofibers with reacted PAMS and reacted PAPS resulted in higher degrees of CaCO$_3$ mineralization than unreacted surfmers. PAPS caused even greater degrees of CaCO$_3$ mineralization than other seeds, including PAMS. Likewise, dye absorption was greatest among the vaterite CaCO$_3$ containing surfaces that were along nanofibers seeded with PAPS. Complexation between PAMS and PVA hydroxyl groups had reduced their ability to attract calcium ions to the surface of nanofibers for mineralization, which also suppressed dye adsorption.

2.2. Introduction

Calcium carbonate (CaCO$_3$) is a naturally abundant, low cost adsorbent\textsuperscript{80} that is widely used in environmental remediation; as in the case of oil-spills\textsuperscript{81}, heavy metal removal\textsuperscript{82}, and dye extraction\textsuperscript{78} from water. When applied to high surface area nanofibers,\textsuperscript{1} CaCO$_3$ coatings could enhance their performance in chemical separation.\textsuperscript{2,3}

Several techniques have been used to mineralize polymeric surfaces with CaCO$_3$; hydrothermal mineralization and the cyclical dipping of materials into salt solutions. Calcite, vaterite, and aragonite are crystalline forms of CaCO$_3$. Their formation during the process of CaCO$_3$ mineralization depends upon each technique’s unique set of process parameters.\textsuperscript{83} Common among these techniques is the use of seed particles that can successfully nucleate the growth CaCO$_3$ along a surface. Calcination which transforms precursor into crystals\textsuperscript{84} requires elevated temperatures for crystal formation and can degrade polymers. The hydrothermal approach most resembles natural methods of mineralization. It involves the prolonged exposure of surfaces to a salt solution. Yang et al\textsuperscript{23} immersed chitosan/polyvinyl
alcohol (PVA) nanofibers, seeded with CaCl₂ or CaCO₃ nanoparticles, in aqueous calcium chloride/sodium carbonate (CaCl₂/Na₂CO₃) for up to 40 days at 25 °C. Suslu et al.²⁵ similarly immersed poly(3-hydroxybutyrate-co-3-hydroxyvalerate) nanofibers that were embedded with hydroxyapatite nanoparticles and surfactant into saline solution for 5 weeks at 37 °C. Nanofibers in those studies were seeded with salts and polyelectrolytes to induce mineralization hydrothermally. Carboxylic acids, hydroxyl groups, ether linkages, and sulfate moieties⁸,¹⁰ can adsorb calcium cations (Ca²⁺) for mineralization to occur. When different polymers were blended with poly(acrylic acid) (PAA), PAA carboxylic acid groups attracted Ca²⁺ for CaCO₃ formation.⁸⁵,⁸⁶ Also, PVA hydroxyl groups, coating the surface of commercial polyamide fibers, have incited the nucleation of aragonite or vaterite CaCO₃.²¹

Zhi et al. and Chen et al. mineralized polymeric membranes by alternating their immersion in aqueous solutions of CaCl₂ and Na₂CO₃.⁸⁵,⁸⁶ This technique of mineralization, by alternating dipping solutions, is deemed a more efficient approach to nanofiber mineralization than the hydrothermal method. Nevertheless, the mechanisms underlying natural occurrences of hydrothermal mineralization can inspire the development of synthetic protocols for nanofiber mineralization.

Biomineralization describes the biotic synthesis of highly organized minerals. Calcium-based forms are found in eggshells, teeth, and bones.⁷ Among living organisms, proteins have key roles in the development of mineral phases. The unique secondary structures of these densely charged biomacromolecules often affect the mineralization kinetics.⁷,⁸⁷ The binding of Ca²⁺ to anionic proteins can nucleate mineralization. Further, highly organized structures of protein β-sheets and self-assemblies of amphiphilic protein can influence the morphology of these minerals.⁸⁸ For instance, mollusk have highly organized layers of CaCO₃ crystals
within their shell. Water-insoluble silk fibroin form layered assemblies with water-soluble proteins, so that hydrophobic interactions are maximized. Ca\(^{2+}\) and CO\(_3\)\(^{-}\) ions then interact with acidic functional groups along the water-soluble proteins to yield crystalline CaCO\(_3\). Bone consists of hydroxyapatite (Ca\(_5\)(PO\(_4\))\(_3\)(OH)), which is attached to collagen fibrils. Like the mollusk shell, collagen fibrils (of 50-200 nm) in bone are mineralized at the sites of negatively charged proteins, which are laced throughout its structure. Ping et al highlighted the use of peptides towards mineralizing collagen. Peptides adsorbed within the voids of collagen fibrils and formed amorphous calcium phosphate layers that crystalized. A part of their designed peptides can coordinate a Ca\(^{2+}\) within amorphous calcium phosphate thus reduces transformation energy of amorphous phase to crystalline phase.

Proteins can denature or foul, which are challenges to their use in industrial products. However, surfactants are synthetic alternatives to seeding nanofibers with proteins and other materials (such as polyelectrolytes and salts) for mineralization. Surfactants have a hydrophilic head group and hydrophobic tail. At the critical micelle concentration and above, surfactants can form micelles that are comparable to proteins and their assemblies in size. Li et al treated the surface of PET films with spherical micelles of 1-2 \(\mu\)m. Those micelles later served as templates for CaO\(_3\) mineralization. Suslu et al dispersed hydroxyapatite nanoparticles (HApNP) within poly(3-hydroxybutyrate-co-3-hydroxyvalerate), PHBV, nanofibers using different surfactants, and then mineralized their surfaces with HAp in simulated body fluid. Sodium dodecyl sulfate (SDS) incited the densest form of HAp mineralization along nanofibers versus sodium deoxycholate and Tween 20. Sodium deoxycholate has a rigid steroidal structure. Tween 20 has a branched structure of four...
flexible chains. Differences in nanofiber mineralization were therefore attributed to the surfactant structure.

In this study, we explore the novel seeding of surfmers, i.e. reactive surfactants, within nanofibers for CaCO$_3$ mineralization. Like surfactants, surfmers form micelles above the CMC. Unlike surfactant-micelles, surfmer-micelles can undergo polymerization to form stable particles. Two surfmers were evaluated in this study: polyoxyethylene-1-(alkyloxymethyl) alkyl ether sulfuric ester ammonium salt (PAMS) and polyoxyethylene alkylphenyl ether ammonium sulfate (PAPS). PAPS and PAMS differ structurally in terms of their hydrophobic tails. PAMS comprises a hydrophobic alkyl chain that is expected to render it more flexible than PAPS, which has a rigid phenyl group. The effect of these structural differences on micelle size, the electrospinning of nanofibers, and nanofiber mineralization were discussed. Further, PVA nanofibers seeded with CaCl$_2$, each surfmer (at a concentration above the CMC), and reacted surfmer were evaluated in terms of the mineralization (by the alternating dipping method) and subsequently dye adsorption. Dye adsorption testing will be used to test the functionality of mineralized nanofibers, and the potential use of these mineralized nanofibers in applications of chemical separation.

2.3. Materials and Methods

2.3.1. Materials

Surfmers: PAMS and PAPS by Monticello Inc. were used in this study (see Figure 2.2a,b). Potassium persulfate (K$_2$S$_2$O$_8$), sodium bisulfite (NaHSO$_3$), 2-hydroxylethyl acrylate (HEA comonomer in Figure 2.2c), CaCl$_2$, Na$_2$CO$_3$, acetone, 70% glutaraldehyde (GA) in water, 1 N sulfuric acid (H$_2$SO$_4$) and polyvinyl alcohol (PVA) were used as-received from Sigma Aldrich®. PVA, having 88% hydrolysis, had a molecular weight of 130 kDa.
2.3.2. Methods

2.3.2.1. Micellar Particle Formation & Polymerization

The CMC for each surfmer was determined by conductivity and surface tension measurements (Appendix A, Figure A1). At 0.1 wt % surfmer in water, aqueous solutions were above their CMC, which is necessary for micelle polymerization.90

To react each type of surfmer-micelle, 0.1 g of surfmer (PAMS or PAPS) in 50 mL of water was added to the reaction flask and maintained at 60 °C under N2 purging. Afterwards, HEA and redox initiators (sodium bisulfite and potassium persulfate91) were added dropwise through separate syringe tips for 3 h. The molar ratio of initiator: surfmer: comonomer was 1:10:10. The addition of comonomer was necessary for surfmer-micelles to polymerize.

Figure 2.2d illustrates the proposed structure of micellar particles before and after polymerization. Polymerization of the micelles was confirmed using proton nuclear magnetic resonance spectroscopy (1H NMR) on the Varian Gem 2300 300 MHz spectrometer for 128 scans. Reacted micelle solutions were dried and re-dissolved in deuterated DMSO (d6 DMSO) prior to 1H NMR. 1H NMR peaks of unreacted surfmer were estimated by ChemBioDraw Ultra 14.0. These computational estimates were used to assign peaks among experimental spectra. 1H NMR spectra of unreacted and reacted are shown in Figure 2.3. After polymerization, peaks at 5.2-5.0 ppm representing H2C=C- in PAMS disappeared. Peaks at ~2.0 ppm disappeared for -HC=CHCH3, and peaks at 6.5-6.6 ppm disappeared for -HC=CHCH3 in PAPS. After reaction, the intensities of peaks representing ethoxylated segments were relatively stronger than the intensity of -CH2- groups along PAMS. Further, after reaction, the intensities of peaks representing ethoxylated groups were relatively stronger than the intensity of -C9H19 and phenyl ring peaks from PAPS. The surfmer
concentration within NMR samples was between 2-10 mg/mL, i.e. 0.2-1 wt% which is above the CMC. Encapsulation of surfmer hydrophobic groups within the micelle core would cause the reduction of -(CH\_2)\_n- peaks among PAMS and PAPS post reaction. For the same reason, aromatic carbons also disappeared post reaction. Zhu et al\textsuperscript{92} reported similar phenomenon after polymerization of micelles also attributed to the restriction of mobility of the surfactant tails.

**Figure 2.2.** These are the chemical structures of (a) PAMS (b) PAPS, and (c) HEA. The schematic represents surfmer-micelles before and after polymerization in (d). Reacted micelles contained PAPS or PAMS with comonomer HEA, whereas unreacted micelles did not contain HEA.
Figure 2.3. $^1$H NMR spectra of unreacted and reacted (a) PAMS and (b) PAPS.

Surfmer micelles were measured by dynamic light scattering (DLS, on the Malvern Zetasizer Nano ZSP) before and after polymerization. DLS results were included in Figure 2.4. In our study, PAMS micelles (without comonomer) had polymodal sizes distributions. PAMS micelles (without comonomer) were mostly $3 \pm 1$ nm and $242 \pm 46$ nm in size.
Reacted PAMS micelles were unimodal in size. PAMS from smaller micelles or free PAMS surfmers appear to have migrated into larger micelles (more than $10^2$ nm) in size. After polymerization, reacted PAMS micelles averaged $133 \pm 26$ nm. PAPS micelles (without comonomer) were mostly greater than $10^2$ nm in size before reaction; only a fraction of micelles was less than $10^2$ nm in size. After polymerization, reacted PAPS micelles had had narrower distributions of particle sizes that were on the order of $10^2$ nm and multiple particle distributions that were less than 50 nm in size.

At 0.1-1 wt% surfmer, a greater percentage of smaller micelle particles are observed than at the CMC of 0.01 wt% (Appendix A, Figure A2). The addition of hydroxyethylacrylate comonomer and micelle polymerization stabilized particle size near 100 nm. The distribution of reacted PAMS and reacted PAPS micelles were narrower and ~100 nm; however smaller reacted PAPS micelles were also observed. At 0.1-1 wt% surfmer, aliphatic PAMS surfmers more readily formed a narrower distribution of stable particles that were ~100 nm in size. Likewise, the distribution of reacted PAPS micelles were than narrower than unreacted PAPS micelles. Perhaps due to their rigidity, micelle particle size distributions also included much smaller particles at less than 50 nm.

After 50% dilution, particle sizes of $10^2$ nm remained; but their distributions appeared to be broader. Swelling of the hydrophilic micelle corona could broaden the range of micelle sizes. Only a minor phase of smaller particles appeared after dilution. Upon reaction, the surfmer-micelle may comprise of an assembly of reacted oligomers opposed to a continuous chain of polymerized surfmers. The micellar aggregation of surfmer-based oligomers is described as polysoap by Summers & Eastoe (Figure 2.2). In summary, polymerization of
surfmern-micelles did render particles stable and helped to minimize surfmer migration upon dilution.

![Figure 2.4](image.png)

Figure 2.4. The particle size analysis of 0.1 wt% surfmer in water is shown for micelles of unreacted and reacted PAMS and PAPS. Solutions were also diluted by 50%.

2.3.2.2. Electrospinning Solutions

CaCl$_2$, unreacted, and reacted micelle particles were added to spinning solutions of PVA. Each type of seed was added at 0.1 g to 100 mL of PVA solution under constant stirring. The concentration of PVA in aqueous spinning solutions was 12 wt %. The conductivity of each spinning solution was measured by Okatan® EC Tester 11+ Multi Range Conductivity Tester by Eetech Instruments.

2.3.2.3. Electrospinning

Electrospinning was used to prepare nanofibrous membranes from polymer solutions. Solutions were electrospun with a positive voltage bias of 15 or 45 kV, tip to collector distance of 8-10 cm, at room temperature, and 40-50 RH%. Nanofibers were spun onto aluminum mesh.
2.3.2.4. Crosslinking Nanofibers

PVA-based nanofibers were crosslinked with GA to prevent their dissolution in water. Nanofibers were cut into 4 x 5 cm\(^2\) specimens and immersed into 300 mM GA in acetone for 24 h. One droplet of sulfuric acid catalyzed the crosslinking reaction. After immersion, webs were air dried.

2.3.2.5. CaCO\(_3\) Mineralization of Nanofiber

Crosslinked nanofibers of 4 x 5 cm\(^2\) were mineralized with CaCO\(_3\) hydrothermally at room temperature. These nanofibers were immersed in aqueous CaCl\(_2\)/Na\(_2\)CO\(_3\) for 1 week. The alternating dip method for nanofiber mineralization was performed in 4 steps. Nanofibers were dipped in 1) 100 mM of aqueous CaCl\(_2\) for 30 s, 2) rinsed with water for 30 s, 3) dipped in 100 mM aqueous Na\(_2\)CO\(_3\) solution for 30 s, and 4) rinsed with water again. This 4-step cycle was repeated 10-20 times at room temperature (22±1°C).

2.3.2.6. Nanofiber Characterization

Nanofibers were imaged by field emission scanning electron microscopy (FE-SEM, FEI, Verios 460L). Using Image J software, the average diameter of at least 25 nanofibers was measured from SEM micrographs. The morphology of surface mineralized nanofibers was also imaged with SEM. X-Ray photoelectron spectroscopy (SPECS system with PHOIBOS 150 Analyzer) was used to analyze the surface chemistry of electrospun nanofibers. Species of interest were carbon (C 1s), calcium (Ca 2p), chloride (Cl 2p), and sulfur (S 2p). The Nomadic\(^\text{TM}\) Raman Microscope by Bay Spec was used to analyze the crystalline phases of CaCO\(_3\) coatings. Raman spectra were collected using 532 nm laser at 150 mW power, 10X objective lens, 20 scans and 1 sec exposure time.

CaCO\(_3\) growth was quantified in terms of mineralization degree (MD) (see Equation 2.1),
\[ MD = \frac{W_m - W_o}{W_o} \times 100 \quad (2.1) \]

where \( W_m \) is mass of mineralized nanofibers and \( W_o \) is mass of untreated nanofibers that were 2 x 2 cm\(^2\) in size. Three samples were taken from each web. Afterwards, the dry mass was weighed.

Dye removal from water by CaCO\(_3\) provided an indirect measurement of mineralization. Nanofibrous membranes, having dimensions of 2 x 2 cm\(^2\), were immersed in 15 mL of aqueous Acid Blue 25, an anthraquinone dye, at 10 mg/L for 24 h. Three measurements of dye analyte were tested by ultraviolet light spectroscopy with a Varian Cary 300 spectrophotometer. Dye absorbance at \( \lambda_{\text{max}} \) = 602 nm were converted to concentration, using a calibration curve for Acid Blue 25 at concentrations of 1-10 mg/mL. Dye removal was calculated according to Equation 2.2:

\[ \text{Removal} (\%) = \frac{C_o - C_t}{C_o} \quad (2.2) \]

where \( C_o \) is initial dye concentration and \( C_t \) is the time-dependent dye concentration after treatment.

2.4. Results and Discussion

2.4.1. Seeding on Nanofiber Morphology

Beadless nanofibers, with and without seeding, were successfully prepared. Seeded nanofibers were finer than neat PVA fibers (Table 2.1). SEM images of nanofibers are shown in Figure 2.5. The conductivities of polymer solutions were measured to understand this behavior.

Ca\(^{2+}\) and Cl\(^{-}\) ions greatly increased the conductivity of the PVA solution in comparison to unseeded PVA solution, whose values were 2363 ± 121 and 523 ± 2 \( \mu \)S, respectively. Relative to CaCl\(_2\), PAMS and PAPS surfmers did not dramatically affect solution
conductivity. And yet, fine fibers, having average diameters of 105-135 nm, were obtained from PVA seeded with unreacted and reacted surfmers. Reacted and unreacted surfmer-micelles of PAPS slightly increased the conductivity of PVA solutions. Reacted and unreacted micelles of PAMS decreased the conductivity of PVA to average values of 446 and 420 µS, respectively. Nagarajan and Kalpakci found: hydrophilic, nonionic polymer and surfactant micelles can form polymer-micelle complexes in water. PVA/micelle complexes are likely to occur between the –OH groups of PVA and micelles. Thus, solution conductivity would decrease below the value of aqueous PVA. The flexibility of PAMS surfmer may have allowed charge transfer between PAMS micelles and PVA more easily than micelles from the more rigid PAPS surfmer. Therefore, the flexibility of the hydrophobic spacer between the hydrophilic head group and vinyl group could have affected PVA-micelle interactions.

Table 2.1. Effect of Seeding on the Solution Conductivity and Size of Nanofibers from Aqueous PVA Solutions

<table>
<thead>
<tr>
<th>Nanofiber Description</th>
<th>Composition of Electrospinning Solutions</th>
<th>Solution Conductivity [µS]</th>
<th>Fiber Diameter [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVA</td>
<td>12 wt % PVA</td>
<td>523 ± 2</td>
<td>186 ± 116</td>
</tr>
<tr>
<td>CaCl₂/PVA</td>
<td>0.1 wt% CaCl₂ / 12 wt% PVA</td>
<td>2363 ± 121</td>
<td>133 ± 37</td>
</tr>
<tr>
<td>PAMS/PVA</td>
<td>0.1 wt% Unreacted PAMS / 12 wt% PVA</td>
<td>420 ± 33</td>
<td>118 ± 53</td>
</tr>
<tr>
<td>Reacted PAMS/PVA</td>
<td>0.1 wt% Reacted PAMS / 12 wt% PVA</td>
<td>446 ± 25</td>
<td>129 ± 47</td>
</tr>
<tr>
<td>PAPS/PVA</td>
<td>0.1 wt% Unreacted PAPS / 12 wt% PVA</td>
<td>549 ± 10</td>
<td>135 ± 40</td>
</tr>
<tr>
<td>Reacted PAPS/PVA</td>
<td>0.1 wt% Reacted PAPS / 12 wt% PVA</td>
<td>540 ± 3</td>
<td>105 ± 35</td>
</tr>
</tbody>
</table>
Figures 2.5. The electrospun morphologies of untreated (a) PVA, (b) CaCl$_2$/PVA, (c) PAMS/PVA, (d) reacted PAMS/PVA, (e) PAPS/PVA, and (f) reacted PAPS/PVA nanofibers are shown.

Increases in solution conductivity decreased the average diameter of fibers containing CaCl$_2$. Salt addition increased the charge density along the polymer jet, which in turn elongated those jets into finer fibers. Surfers affected solution conductivity less than CaCl$_2$. Nevertheless, surfers resulted in fibers finer than neat PVA. Like nonpolymerizable surfactants, surfers can lower the surface tension of polymer solutions. As a result, finer fibers are expected for fibers spun at similar voltage.

XPS was used to observe evidence of seed particles at the surface of nanofibers. XPS C 1s peaks for seeded nanofibers are shown in Figure 2.6. C 1s peaks consists of C-C (284.8 eV), C-O (286 eV), and C=O (289 eV). The ratio of C-C to C-O was 3.3 for PVA, 2.6 for CaCl$_2$/PVA, 0.9 for PAPS/PVA and 0.8 for reacted PAPS/PVA. Considering that the ratio of C-C to C-O was calculated as 1.9 for 88% hydrolyzed PVA molecules, most –OH groups resided within the core of PVA nanofibers. CaCl$_2$/PVA nanofibers had a higher concentration of C-O groups at their surface in comparison to PVA. The high conductivity of the
CaCl\textsubscript{2}/PVA solution appears to promote the surface migration of –OH groups during electrospinning. Differences between the surface chemistry of nanofibers with and without micelles were caused by the placement of ethoxylated surfmer head groups at the nanofiber surfaces. Polymerized micelles also contained HEA comonomer which could also reduce the C-C to C-O ratios. Reacted PAPS/PVA nanofibers had a slightly higher quantity of C-O groups at its surface than PAPS/PVA nanofibers. Therefore, the micellar structure and composition of reacted PAPS would yield lower C-C to C-O ratios than PAPS/PVA nanofibers.

High resolution XPS scans detected calcium and chloride binding energies for CaCl\textsubscript{2}/PVA nanofibers and sulfur binding energies for PAPS/PVA and reacted PAPS/PVA nanofibers (Figure 2.7). Calcium and chlorine ions were detected at the CaCl\textsubscript{2}/PVA nanofiber surface. These ions can act as nucleating sites. Further, PVA hydroxyl group at the nanofiber surface can also concentrate Ca\textsuperscript{2+} ions to initiate surface mineralization. Similar amounts of sulfur were detected at the surface of PAPS/PVA and reacted PAPS/PVA surfaces. The PAPS surfmer has a hydrophilic head group comprising sulfite and ethoxy groups; wherein, ethoxy groups were detected in greater abundance. PAPS from unreacted surfmer-micelles may be more prevalent at the PVA nanofiber surface than reacted PAPS to give a slightly higher spectral intensity of S. Based on XPS analysis, Figure 2.8 illustrates the surface chemistry of seeded nanofibers.
Figure 2.6. XPS C 1s spectra of untreated (a) PVA, (b) CaCl₂/PVA, (c) PAPS/PVA and (d) reacted PAPS/PVA nanofibers along with deconvoluted peaks are shown.
Figure 2.7. High resolution scans of XPS spectra for (a) Ca 2p and (b) Cl 2p in CaCl$_2$/PVA nanofibers, (c) S 2p in PAPS/PVA and (d) S 2p in reacted PAPS/PVA.

Increased amounts of hydroxyl groups at the CaCl$_2$/PVA nanofiber surface were attributed to concentrating Ca$^{2+}$ ions on the surface of mineralized nanofibers. The availability of charge at the nanofiber surface is expected to influence CaCO$_3$ mineralization. Anionic species are expected to nucleate mineralization by attracting Ca$^{2+}$ ions to the nanofiber surface. Under positive voltage bias, ionic salts and micelles are expected to migrate towards the surface of nanofibers. Surfactants$^{25}$, proteins$^{59}$, and polymer functional groups$^{60}$ are manipulated towards the surface of electrospun nanofibers per high voltage and pH adjustments. Differences in electrical permittivity constants can also result in
dielectrophoretic migration within the non-uniform electric field.\textsuperscript{59,61} Surface migration of these ionically-charged, seed particles can also occur as nanofibers are immersed in water.\textsuperscript{100}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.8}
\caption{The surface chemistry of seeded nanofibers is illustrated, as evidenced by XPS.}
\end{figure}

2.4.2. Mineralization by Alternating Dipping Versus Immersion

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.9}
\caption{Morphologies of CaCO\textsubscript{3} mineralized PAMS/PVA nanofibers by alternating dip cycles of aqueous CaCl\textsubscript{2} and Na\textsubscript{2}CO\textsubscript{3} are shown for (a) 10, (b) 15, and (c) 20 cycles. Nanofibers were dipped into CaCl\textsubscript{2}/Na\textsubscript{2}CO\textsubscript{3} solution for 1 week (d). CaCO\textsubscript{3} mineralized CaCl\textsubscript{2}/PVA nanofibers by alternating dip cycles for (e) 10, (f) 15, and (g) 20 cycles are shown.}
\end{figure}

Spherical CaCO\textsubscript{3} particles produced by the alternating dip method enveloped the circumference of nanofibers seeded with PAMS and CaCl\textsubscript{2} (Figure 2.9). As dipping cycles increased, CaCO\textsubscript{3} particles also increased in size around nanofibers. The size and shape of CaCO\textsubscript{3} particles were most homogeneous after 10 cycles (Figure 2.9a, e). After 15 dipping
cycles, rhombohedral CaCO$_3$ particles had grown among the spherical particles although much fewer in number (Figure 2.9b, f). Nanofibers immersed in aqueous CaCl$_2$/Na$_2$CO$_3$ for 1 week had shown irregular CaCO$_3$ particles of aggregated parallelograms, where CaCO$_3$ precipitated onto the nanofiber surface. 10 alternating dip cycles were shown to successfully mineralize PVA nanofibers and yield a desirable morphology of spherical CaCO$_3$ surrounding nanofibers.
2.4.3. Effects of Polymerized Micelles on Mineralization

Figure 2.10. Mineralized (a) PVA (b) CaCl\(_2\)/PVA (c) PAMS/PVA (d) reacted PAMS/PVA (e) PAPS/PVA and (f) reacted PAPS/PVA nanofibers are shown. These fibers were mineralized by alternating dipping cycles ten times.

Micron-sized, CaCO\(_3\) spheres engulfed portions of neat PVA and seeded nanofibers (Figure 2.10). PVA hydroxyl groups may have allowed nucleation along the fiber circumference. In several instances, spherical CaCO\(_3\) particles were adjacently aligned along individual fibers—especially among nanofibers seeded with PAMS and PAPS. CaCO\(_3\) particles along each of the CaCl\(_2\), unreacted, and reacted PAMS seeded nanofibers appear
uniform in size. Nanofibers seeded with unreacted and reacted PAPS show a mixture of 
CaCO₃ particle sizes. To further understand effects of seeding on CaCO₃ morphology, Raman 
spectroscopy and dye adsorption tests were conducted.

Raman peak intensities between 1050-1100 cm⁻¹ were associated with CaCO₃ phases⁸⁴,¹⁰¹ among mineralized nanofibers (Figure 2.11). Raman peaks at ~1085, 712, and 284 are attributed to symmetric stretching (ν₁), in-plane bending (ν₄), and lattice modes of calcite, respectively.⁸³,⁸⁴,¹⁰² Mineralized PVA had broad, weak signals within this region, which is indicative of low CaCO₃ crystal formation.⁸⁴ Electron donating hydroxyl groups along PVA can nucleate CaCO₃ formation; however crystalline CaCO₃ was not achieved. Raman intensity peaks were not observed at ~284 and 712 cm⁻¹ which also represent calcite CaCO₃.

Nanofibers seeded with CaCl₂ yielded some calcite CaCO₃, as noted by the sharp peak at 1086 cm⁻¹. Nanofibers seeded with PAMS, PAPS, reacted PAMS, and reacted PAPS yielded some vaterite CaCO₃, as noted by peaks at 1078 and 1091 cm⁻¹.¹⁰³ Raman intensities shouldered 1086 cm⁻¹, which suggests calcite CaCO₃ was formed among these coatings. Seeding seemed to promote crystalline CaCO₃ phase formation at the nanofiber surface. Especially, unreacted and reacted PAMS or PAPS micelles developed some vaterite phase which is not the thermodynamically stable state. Thus, it is plausible to assume that surfmers not only provide nucleating sites but also act as crystal growth modifier as Li et al proposed.²⁶ Our results from Raman spectroscopy are in line with SEM morphology in that previous studies reported spherical calcite and vaterite structures. It is noteworthy that reacted PAMS and reacted PAPS showed stronger peak intensities than unreacted surfmers. Reacted PAPS had the strongest crystal peaks- indicating its rigid micelle structure and high charge density was most effective in promoting CaCO₃ nucleation and growth.
Figure 2.11. Raman spectra from mineralized nanofibers of PVA with and without seed particles. Untreated PVA indicates crosslinked PVA nanofibers before mineralization.

2.4.4. Mineralized Nanofibers as Dye Adsorbents

CaCO₃ mineralization of nanofibers were tested according to their ability to remove dye from water (Figure 2.12 and Table 2.2). The 2 x 2 cm² nanofibrous webs were ~1 mg before mineralization. Seeding nanofibers with CaCl₂ or micelles yield higher MD values than PVA. Ionic salts and micelles attracted Ca²⁺ ions to the surface of nanofibers. Yang et al.²³ proposed that Ca²⁺ ions distribution at fiber surfaces affect CaCO₃ crystal nucleation. Reacted PAPS/PVA had the highest MD value which again shows the effectiveness of reacted PAPS in promoting CaCO₃ mineralization. Mineralized nanofibers adsorbed anthraquinone Acid Blue 25 from water, as shown by stained webs. Dye uptake in (unmineralized) neat PVA nanofibers is attributed to swelling. PVA is a hydrophilic polymer. Crosslinking helps to prevent dissolution, nevertheless it is expected to swell in water. Although adsorption between -OH groups and dye is possible, the photograph of PVA nanofibers in dye solution
does not show the level of staining that is observed among mineralized nanofibers. Therefore, update of dye in PVA nanofibers was caused by swelling. Dye adsorption was visually observed among the stained nanofibers that were coated with CaCO$_3$. Dye adsorption was greatest among nanofibers seeded with CaCl$_2$, reacted PAMS, PAPS, and reacted PAPS. These nanofibers also showed the highest degrees of mineralization (MD in Table 2.2). Mineralized PAMS/PVA had similar dye adsorption as mineralized PVA regardless of its lower MD value. Strong interactions between PAMS and PVA-OH could lend PAMS less accessible to ions for mineralization than PAPS. This complex would also lend PVA-OH groups less accessible for moisture adsorption. Reacted PAPS yielded the highest value of MD and dye from water removal. Photos of dye adsorption by mineralized PAPS/PVA and reacted PAPS/PVA were darker than other mineralized nanofibers. Therefore, the seeding of nanofibers with polymerized micelles is a viable strategy for mineralizing functional nanofibers.

**Figure 2.12.** Photograph of Acid Blue 25 adsorbed by mineralized (a) CaCl$_2$/PVA nanofibers revealed the checkered pattern of nanofibers electrospun onto aluminum mesh. The structure of the (b) anthraquinone dye is shown. The contrast in dye absorbed from 10 mg/L solutions is shown for different samples of mineralized nanofibers (c).
Table 2.2. Dye Adsorbed by Mineralized Nanofibers Containing Different Seeds from 10 mg/L Acid Blue Dye Solutions. Untreated PVA indicates crosslinked PVA nanofibers before mineralization.

<table>
<thead>
<tr>
<th></th>
<th>Untreated PVA</th>
<th>PVA</th>
<th>CaCl2/PVA</th>
<th>PAMS/ PVA</th>
<th>Reacted PAMS/ PVA</th>
<th>PAPS/ PVA</th>
<th>Reacted PAPS/ PVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basis weight (g/m²)</td>
<td>2.8</td>
<td>2.8</td>
<td>2.8</td>
<td>2.8</td>
<td>2.8</td>
<td>3.0</td>
<td>1.8</td>
</tr>
<tr>
<td>Mineralization degree (%)</td>
<td>-</td>
<td>97</td>
<td>218</td>
<td>61</td>
<td>148</td>
<td>258</td>
<td>348</td>
</tr>
<tr>
<td>Dye removal (%)</td>
<td>23</td>
<td>36</td>
<td>45</td>
<td>33</td>
<td>45</td>
<td>44</td>
<td>52</td>
</tr>
<tr>
<td>Adsorption (mg/m²)</td>
<td>58</td>
<td>90</td>
<td>112</td>
<td>84</td>
<td>112</td>
<td>111</td>
<td>130</td>
</tr>
</tbody>
</table>

* Basis weight and dye removal was within ± 1 % error
* Mineralization degree was within ± 10 % error

2.5. Conclusions

Polymerized micelles were successfully used to seed nanofibers for CaCO₃ mineralization. Negatively charged seeds of surfmer-micelles migrated towards fiber surfaces during the process of electrospinning. At fiber surfaces, seeds concentrated Ca²⁺ and CO₃²⁻ ions during mineralization by alternating cycles of dipping. Reacted PAMS and reacted PAPS resulted in spherical CaCO₃ clusters and the highest degrees of mineralization. Reacted PAPS resulted in higher degrees of CaCO₃ mineralization along nanofibers than reacted PAMS, presumably due to its more rigid structure. Surfmer structure influenced the availability of surfmer micelles within the PVA nanofibers to nucleate the growth of CaCO₃ from its surface. Micelles of unreacted and reacted PAMS were deemed more flexible than micelles of unreacted and reacted PAPS. Thus, the ionic charges on PAMS micelles were more likely
to complex with PVA -OH groups and remain shielded from Ca\(^{2+}\) ions. Surfmere-micelles complexed with PVA -OH would also hinder moisture adsorption by the matrix polymer.

Raman spectroscopy revealed the influence of seeding on the crystalline morphology of mineralized nanofibers. Micelles acted as both nucleating sites and crystal growth modifier to yield partial vaterite phases which can be critical for adsorbent design. Previous study showed that formaldehyde adsorption is promoted by vaterite phases.\(^{79}\) The polymerization of surfmeme-micelles resulted in stable supramolecular structures that exhibit high charge density. Thus, polymerized micelles are supramolecular alternatives to proteins, surfactants and salts in promoting mineralization. The observations in this study are causes for further investigation, as are the influence of other micelle structures on the mineralization and functionality of nanostructured coatings.
CHAPTER 3

TITANIUM OXIDE SOL-GEL INDUCED WRINKLING OF ELECTROSPUN NANOFIBERS

3.1. Abstract

![Figure 3.1](image)

**Figure 3.1.** Graphical abstract of Chapter 3.

TiO$_2$ sol-gel reaction induced nanowrinkling along the surface of electrospun poly(vinyl alcohol) (PVA) nanofibers. Nanowrinkling along nanofibers was influenced by electrospinning voltage, degree of nanofiber crosslinking, and the repetitive immersion of nanofibers in TiO$_2$ precursors. Crosslinked nanofibers were dipped in alternating solutions of titanium tetraisopropoxide (TTIP) and water for up to 5 cycles. Interestingly, nanowrinkles only formed along nanofibers that were spun at 45 kV and treated with 3 or more cycles of sol-gel precursor. Spectroscopy revealed: More PVA hydroxyl groups populated the nanofiber surface when electrospinning occurred at 45 kV than at 15 kV. In turn, PVA hydroxyl groups on surfaces appeared to nucleate TiO$_2$ growth. Scanning probe phase

---

2 The material in this chapter was submitted as: Park Y, Ford E. Titanium Oxide Sol-Gel Induced Wrinkling of Electrospun Nanofibers. *Macromolecular Chemistry and Physics.*
micrographs confirmed modulus differences between the attached TiO$_2$ particles and PVA’s surface. Those moduli differences result in wrinkling, as TTIP treated nanofibers undergo repetitive cycles of water swelling and isopropanol deswelling.

3.2. Introduction

Advanced materials having wrinkled morphologies are potentially useful as microfluidic channels\textsuperscript{104}, separation membranes\textsuperscript{34}, superhydrophobic surfaces\textsuperscript{105,106}, and electrodes\textsuperscript{107}. Flow channels, as produced by wrinkling, can tune surface wetting in those applications. In the case of materials designed for energy storage, wrinkling shortened pathways for electron flow, while increasing the surface area of conductive material.\textsuperscript{107}

Wrinkling is synthetically induced upon the relaxation of pre-strained multilayers.\textsuperscript{34,107–109} The stiff inorganic layer is physically deposited onto the soft material surface. For example, gold (Au) multilayers were deposited onto the surface of pre-stretched films of polydimethylsiloxane (PDMS)\textsuperscript{108} or polystyrene (PS)\textsuperscript{107}, which wrinkled upon relaxation. Strain differences between the gold skin and polymeric surface produced wrinkling. The chemical modification of polymeric substrates by oxidative radicals and ionization also stiffens their surface. Ultraviolet/ozone (UVO) treatment\textsuperscript{34} and oxygen gas derived plasma\textsuperscript{109} formed a stiff silicon oxide skin along the surface of PDMS.

Layered polymeric films, wherein each layer has a different coefficient of thermal expansion, will wrinkle upon heating\textsuperscript{33,110}. Okayasu et al.\textsuperscript{110} observed the wrinkling of aluminum-polystyrene-silicon (Al-PS-Si) thin films after a cycle of thermal annealing and cooling. Si, whose thermal coefficient of expansion was an order of magnitude lower than that of Al-PS, stymied the thermal expansion of the multilayered film when heated above the glass transition temperature of PS. Recently, Wang et al.\textsuperscript{33} induced wrinkles along
polydopamine/polystyrene (PDA/PS) bilayers with thermal annealing at 120 °C. Wrinkling occurred at elevated temperature because PDA’s thermal coefficient of expansion was lower than that of PS.

Swelling among copolymer thin films that were anchored to rigid surfaces has led to wrinkling.\textsuperscript{111,112} Trujillo et al\textsuperscript{111} reported the wrinkling of poly(acrylamide-co-sodium acrylate) hydrogels that were attached to glass. The hydrogel swelled vertically in phosphate-buffered saline (PBS) solution but lateral swelling was restricted by its adhesion to glass. Wrinkling only occurred once vertical swelling reached a critical point- when thickness changed by a factor of 2. Thermally responsive, crosslinked poly(N-isopropylacrylamide-co-sodium acrylate) hydrogels were attached to rigid glass, which led to stimuli-responsive wrinkling.\textsuperscript{112} The poly(N-isopropylacrylamide) (NIPAM) copolymer gel is a well-known temperature-responsive polymer. As NIPAM is heated in water above its lower critical solution temperature (LCST), it expels water from the swollen gel as intramolecular hydrogen bonding is disrupted. Deswelling of anchored hydrogel led to wrinkling at 33-37 °C.

Artificially-induced wrinkling resembles naturally occurring wrinkles, as found among bacterial biofilms and along human skin.\textsuperscript{28} Bacterial cells are embedded within the extracellular matrix (ECM) of biofilms. Structural proteins, polysaccharides, and carbohydrates comprise the ECM. Biofilm wrinkling is caused by the localization of stiff dead cells that reside among new cells and ECM. Dead cell regions act as focal points of wrinkling.\textsuperscript{29} The wrinkling of human skin results from similar processes. Skin consists of two main layers: epidermis and dermis. Epidermis is an outer-layer of mostly dead cells, while the dermis sublayer has living cells throughout its ECM.\textsuperscript{30} Wrinkling occurs with aging, as
skin’s outer layer stiffens relative to inner layers and as skin repeatedly undergoes muscular tightening and relaxation. The loss of elastic collagen and elastin fibers from skin’s outer layer are other causes of stiffening.

Up to now, artificially induced wrinkling has resulted from thin film preparation or anchored gels. Herein, we report the novel finding of nanowrinkling along electrospun nanofibers during sol-gel synthesis. Crosslinked poly(vinyl alcohol) (PVA) nanofibers were immersed in TiO$_2$ sol-gel precursor- titanium isopropoxide (TTIP) which hydrolyzes into titanium hydroxide (Ti(OH)$_4$). To the authors’ knowledge, this is the first time that fine, uniaxially aligned wrinkles formed as nanofibrous membranes mineralized. The uniaxially aligned nanowrinkles, as reported by Kim et al., formed along hydrogels anchored to rigid substrates. In this research, surface-active Ti(OH)$_4$ confined regions of crosslinked PVA surfaces, which then resulted in wrinkling and TiO$_2$ mineralization. TiO$_2$ sol-gel synthesis evolved from the cyclical immersion of nanofibers in alternating solutions of TTIP and water. The alternating dipping method for sol-gel synthesis was used by Park et al. to mineralize the surface of electrospun PVA with calcium carbonate (CaCO$_3$). Applied voltage for electrospinning, degree of nanofiber crosslinking, and cycles of TTIP immersion played key roles in dictating wrinkle formation.

3.3. Materials and Methods

3.3.1. Materials

PVA, acetone, isopropyl alcohol (IPA), 50% glutaraldehyde (GA) in water, 1 N sulfuric acid (H$_2$SO$_4$), and titanium tetraisopropoxide (TTIP) were used as-received from Sigma Aldrich$^\text{®}$. PVA, having 99% hydrolysis, had a molecular weight of 89-98 kDa.
3.3.2. Methods

3.3.2.1. Electrospinning Nanofibers

Aqueous spinning dope of 11 wt% PVA was prepared under constant stirring at 60 °C for 1 h. PVA nanofibers were electrospun at 15 and 45 kV using two different electrospinning set-ups- the lab-scale and pilot-scale techniques are shown respectively in Figure 3.2a and 3.1b. Lab-scale electrospinning at 15 kV was performed using a horizontal tip to collector distance of 8 cm, feed rate of 0.8 mL/h, and 18-gauge syringe needle (inner diameter of 0.838 mm). Pilot-scale electrospinning at 45 kV was performed using a vertical electrode to collector distance of 10 cm on the Elmarco Nanosider. The roller electrode was immersed in polymer solution and rotated at 8 rpm for needless electrospinning. For both set-ups, nanofibers were collected onto aluminum mesh at room temperature and 40-50% relative humidity.

![Figure 3.2](image1.png)

**Figure 3.2.** Photographs show electrospinning on the a) lab-scale to produce 15 kV nanofibers and b) pilot scale to produce 45 kV nanofibers.

3.3.2.2. Crosslinking Nanofibers

PVA nanofibers were crosslinked prior to TiO$_2$ sol-gel synthesis. Aqueous GA was diluted to 300 and 400 mM GA in 50 mL of acetone. One droplet (~50 µl) of sulfuric acid was added as catalyst. Nanofibrous webs of 4 x 5 cm$^2$ were immersed into GA solution at room
temperature for 24 h. After immersion, webs were air dried at room temperature. The acid catalyzed crosslinking of PVA hydroxyl groups with GA is shown in Scheme 3.1a. GA treated PVA results in intermolecular crosslinking and unreacted GA aldehyde groups.\textsuperscript{114,115}

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\includegraphics[width=0.5\textwidth]{scheme1a.png}};
\end{tikzpicture}
\end{center}

\textbf{Scheme 3.1.} (a) Structures of PVA crosslinked with GA and (b) sol-gel synthesis of TiO$_2$ are represented.

### 3.3.2.3. TiO$_2$ Sol-Gel Synthesis

TiO$_2$ was synthesized on the surface of crosslinked PVA nanofibers using the modified sol-gel synthetic approach described in Fischer et al.\textsuperscript{116} The proposed reaction for sol-gel synthesis is shown in Scheme 3.1b.\textsuperscript{117,118} TTIP solution was prepared from 2 mM TTIP in 50 mL IPA. As also performed by Fischer et al.\textsuperscript{116}, crosslinked nanofibers underwent prewetting by solvent in three steps: Step 1) IPA for 30 s, Step 2) 50/50 (volume/volume, v/v) IPA/water for 1 min, and Step 3) water for 2 min to aid the homogenous synthesis of TiO$_2$ along nanofibers. Afterwards, nanofibers were dipped in alternating solutions of TTIP solution for 5
min, then in water for 1 min for up to 5 cycles at room temperature (22±1 °C), and finally air dried for 24 h.

Since IPA and water are used during TiO$_2$ sol-gel synthesis, their role on nanofiber wrinkling and swelling was studied. PVA nanofibers were prewetted in three steps: Step 1) IPA for 30 s, Step 2) 50/50 (v/v) IPA/water for 1 min, and Step 3) deionized water for 2 min. Afterwards, nanofibers were dipped in alternating solutions of IPA for 5 min, water for 1 min for up to 5 cycles at room temperature (22 ± 1 °C), and finally air dried for 24 h.

Fiber swelling was tested among crosslinked PVA fibers. Fibers were immersed in a vial of distilled water or IPA for 24 h at room temperature. Samples were taken from the vials, and excess solvent was absorbed by paper towel. Fiber swelling ratio (S) was calculated using Equation 3.1,

$$S (\%) = \frac{w_w - w_d}{w_d} \times 100$$

(3.1)

where $w_d$ is the dry weight of fibers and $w_w$ is the wet weight of fibers.

3.3.2.4. Image Analysis

The morphology of electrospun PVA was analyzed using field emission scanning electron microscopy (FE-SEM, FEI, Verios 460L) and scanning probe atomic force microscopy (AFM, Bruker Dimension 3000). Chapman et al.\textsuperscript{35} and Gabardo et al.\textsuperscript{107} measured wrinkle wavelength from SEM images. Thus, SEM was used in this study to characterize wrinkles and fiber size. ImageJ analysis of SEM images was used to determine the average diameter of at least 50 nanofibers and the average wrinkling wavelength from 50 wrinkles. AFM height and phase images were collected from tapping mode measurements in air at 1 Hz and 512 samples/line. Budget Sensors Tap 300 AFM tip, having an aluminum reflex coating, 300 kHz resonant frequency, and 40 N/m force constant was used.
3.3.2.5. Spectroscopic Analysis

The Nomadic™ Raman Microscope by BaySpec was used to characterize electrospun nanofibers after TiO$_2$ sol-gel treatment. Raman spectra were collected using the 532 nm laser at 150 mW power, 10X objective lens, 20 scans and 1 sec exposure time. Peaks were normalized by the 2910 cm$^{-1}$ peak which represent C-H stretching of multiple -CH$_2$- groups.$^{119}$

Infrared absorbance spectra were collected on the Nicolet iS50 spectrophotometer at 128 scans and a spectral resolution of 4 cm$^{-1}$. Collected spectra were subjected to baseline subtraction and normalized to the C-H bending peak at 1450 cm$^{-1}$. To quantify PVA crosslinking, acetal bridge formation (C-O-C) at the 1097 cm$^{-1}$ peak was normalized by the 1450 cm$^{-1}$ peak.$^{120}$ An index of PVA’s crystallinity was calculated from the ratio of the 1144 cm$^{-1}$ peak to 854 cm$^{-1}$ peak.$^{121,122}$ The intensity of the peak at 1144 cm$^{-1}$ represents symmetric C=C stretching or the stretching of C-O in the crystalline phase. The band at 854 cm$^{-1}$ (C-C stretching) was chosen as the reference band since its absorbance is not affected by processing.$^{123,124}$

X-Ray photoelectron spectroscopy (SPECS system with PHOIBOS 150 Analyzer) was used to analyze the surface chemistry of electrospun nanofibers. Mg K$\alpha$ (1253.6 eV) source was operated at 10 – 14 kV. Carbon (C 1s) and oxygen (O 1s) peaks were deconvoluted using Gaussian distributions through Origin 2016 software.

3.4. Results and Discussion

3.4.1. 300 mM GA Crosslinked Fiber Morphology After TiO$_2$ Synthesis

PVA nanofibers were electrospun at 15 and 45 kV prior to crosslinking in 300 mM GA solution. The smooth, well-defined nanofibers had an average diameter of 184 ± 59 nm when
Electrospun at 15 kV (see Figure 3.3ai). After crosslinking in aqueous GA, nanofibers swelled in size from 184 ± 59 nm to 255 ± 65 nm; however, their fibrous shape did not change after crosslinking (see Figure 3.3aii). Nanofibers spun at 45 kV had similar morphology and diameter of 191 ± 85 nm (see Figure 3.3bi). After crosslinking, 45 kV nanofiber swelled and formed film like structures (see Figure 3.3bii).

Electrospun nanofibers were dipped in alternating solutions of TTIP and water for up to 5 cycles to synthesize TiO₂ on their surfaces (see Figure 3.3aiii-v, 3.3biiv-v). After 1 dipping cycle, the 15 kV nanofibers retained their distinct fiber morphology and pore structure (Figure 3.3aiii). TiO₂ nanoparticles were observed along fiber surfaces. The 45 kV nanofibers had shown fiber interconnected by film, without any obvious pore structure, after one cycle of dipping (Figure 3.3biii). The 45 kV nanofibers were visually more translucent than the 15 kV nanofibers after sol-gel synthesis (Appendix B, Figure B1). Fewer aggregates of TiO₂ were observed along the 45 kV nanofibers than the 15 kV nanofibers (Figure 3.3aiii, biii). However, 45 kV nanofibers were stiffer than the 15 kV nanofibers. Stiffening from sol-gel synthesis suggests TiO₂ attached itself to polymeric nanofibers and covered the surface of 45 kV nanofibers more uniformly than the 15 kV nanofibers.
Figure 3.3. Micrographs of PVA nanofibers electrospun at (a) 15 and (b) 45 kV are shown (i) before and (ii) after crosslinking with 300 mM GA. Crosslinked nanofibers were dipped in alternating solutions of TTIP and water for (iii) 1 (iv) 3 and (v) 5 cycles.
With 3-5 consecutive cycles of dipping, TiO$_2$ particles aggregated onto the surface of porous nanofibers that were electrospun at 15 kV (Figure 3.3aiii-v). 45 kV nanofibers featured some aggregation among TiO$_2$ particles and a new surface feature of nanowrinkles after 3 cycles of dipping. These wrinkled structures were most prominent after the 5th dipping cycle; by then, pores along 45 kV nanofibers were completely masked.

3.4.2. 400 mM GA Crosslinked Fiber Morphology After TiO$_2$ Synthesis

To understand the role of crosslinking on TiO$_2$ sol-gel synthesis and nanowrinkling, nanofibers spun at 15 and 45 kV were also crosslinked with 400 mM GA solution. The morphology of nanofibers crosslinked with 400 mM GA solution and treated with alternating solutions of TTIP and water are shown in Figure 3.4. PVA nanofibers that were electrospun at 15 kV were smooth and 184 ± 59 nm in diameter. Fiber diameter was not affected by crosslinking in 400 mM GA solution (191 ± 39 nm). However, PVA nanofibers that were electrospun at 45 kV had shown coarse wrinkles and fibers adjoined by film.
Figure 3.4. Micrographs of PVA nanofibers electrospun at (a) 15 and (b) 45 kV and crosslinked with 400 mM GA are shown (i). Crosslinked nanofibers were dipped in alternating solutions of TTIP and water for (ii) 1 (iii) 3 and (iv) 5 cycles. With additional cycles of dipping, TiO$_2$ particulate coatings became denser. TiO$_2$ aggregated along the surface of porous 15 kV nanofibers (Figure 3.4aii-aiiv). 15 kV fibers did not show evidence of swelling and their porosity was preserved. In contrast, 45 kV
nanofibers merged together through film formation. By the 5th cycle, coarse wrinkles became finer and pores were masked. TiO$_2$ particles had grown along the contours of wrinkles (Figure 3.4biii, biv). Nanowrinkles were directed along the axes of distinct fibers and aligned among the thin film regions that connected nanofibers.

### 3.4.3. Characterization of Wrinkling

Nanowrinkles were characterized by electron and scanning probe microscopy. The average wavelength of wrinkles was measured from high resolution SEM images, as shown in (Appendix B, Figure B2 & Table B1). As previously discussed, nanowrinkles were observed along the surface of crosslinked, 45 kV nanofibers that underwent 3-5 cycles of sol-gel dipping. Wrinkling along 45 kV, 400 mM GA fibers (Sample ID describes the applied voltage for electrospinning and the molarity of GA within crosslinking solution) had a smaller wavelength than 45 kV, 300 mM GA fibers- both after 5 dipping cycles. The wrinkling wavelength along 45 kV, 300 mM GA fibers increased from 115 ± 22 nm to 231 ± 42 nm after 3 to 5 dipping cycles for sol-gel synthesis. Wrinkles having a wavelength of 191 ± 32 nm formed along 45 kV, 400 mM nanofibers after 5 dipping cycles.

AFM revealed the hierarchical structure of TiO$_2$ coated nanofibers. According to AFM height profiles, surface roughening was caused by nanowrinkling and TiO$_2$ synthesis (Figure 3.5). For example, the root mean squared (RMS) roughness of the 45 kV, 300 mM fibers increased from 17 to 72 nm after 5 cycles of TiO$_2$ coating. TiO$_2$ particles were along the peak of wrinkles.

AFM phase imaging revealed distinct differences between the surface chemistries of the untreated and sol-gel treated nanofibers. Phase imaging represents the lag between the cantilever’s oscillating frequency and driver, which is caused by cantilever tip interactions.
with the sample. These interactions include intermolecular forces between the tip and sample surface, as well as variations in sample stiffness. Neat PVA nanofibers showed a mostly uniform phase lag; whereas the TiO$_2$, crosslinked nanofibers showed three distinct regions: TiO$_2$ particles, PVA phase bordering TiO$_2$ particles, and the PVA phase that separates TiO$_2$ particles. TiO$_2$ has a higher Young’s modulus (151 GPa) than PVA (up to 80 GPa). Repulsive forces between the scanning probe and relatively stiff surface enhances the phase shifts towards positive bright imaging, while attractive forces results in phase shifts towards negative dark imaging. Therefore, the overall stiffening of 45 kV fibers was due to the nucleation and growth of TiO$_2$ particles along the soft domains of the wrinkled nanofibers. Spectroscopy was used to investigate surface chemistry, as the result of these soft domains of adhesive polymer. These domains are believed to have an important role in TiO$_2$ synthesis along the wrinkled nanofibers.
3.4.4. Role of TiO$_2$ Synthesis on Wrinkling

Sol-gel immersion solvents were tested as potential sources of nanofiber wrinkling. However, the immersion of crosslinked nanofibers in IPA did not induce wrinkling, as shown in Appendix B, Figure B3. Therefore, TiO$_2$ synthesis must have a crucial role in forming...
nanowrinkles along the surface of 45 kV fibers. Based on AFM phase imaging, the surface chemistry of PVA bordering TiO₂ nanoparticles are likely rich in PVA hydroxyl (-OH) groups. In turn, adhesive, hydroxyl-rich regions are capable of nucleating TiO₂ by means of bonding with titanium hydroxide, Ti(OH)₄, intermediates.¹¹⁸

Compared to 15 kV nanofibers, 45 kV nanofibers showed strong Raman peaks at 1072 to 1095 cm⁻¹, which are assigned to saturated secondary alcohols.¹¹⁹ These strong C-O Raman peaks at 1072 cm⁻¹ and 1095 cm⁻¹ relate to amorphous polymer. The intensities of peaks between 1072 cm⁻¹ to 1095 cm⁻¹ decreased as TiO₂ sol-gel cycles increased, because -OH groups within these amorphous regions can interact with TTIP to nucleate TiO₂ along the surface. The -OH peak from 15 kV nanofibers shifted from 3175 cm⁻¹ to 3155 cm⁻¹, and the -OH peak of 45 kV nanofibers shifted from 3165 cm⁻¹ to 3150 cm⁻¹. These trends indicate hydrogen bonding between TiO₂ nanoparticles and PVA -OH groups.¹¹⁹

Amorphous TiO₂ formed along the 45 kV nanofibers, as noted by the low intensity Raman peaks between 100 - 800 cm⁻¹ (Figure 3.6). Incomplete hydrolysis of TTIP as shown by the -CH and -OH bending peak at 1440 cm⁻¹¹²⁸ as well as strong -CH₂- peak at 2880-2980 cm⁻¹¹¹⁹ indicate amorphous TiO₂. Residual TTIP alkoxy groups stem from incomplete hydrolysis and behave as structural impurities that hinder TiO₂ crystallization.¹¹⁸ High temperature calcination is required to transform TiO₂ from the amorphous to crystalline phase.¹²⁹
Figure 3.6. Raman spectra of 15 kV-300 mM GA and 45 kV-300 mM GA nanofibers treated with up to 5 cycles of TiO$_2$ sol-gel coating.

High voltage can drive polar groups towards the electrospun nanofibers’ surface under positive voltage bias.$^{60,130}$ Thus, the 45 kV nanofibers were presupposed to have more -OH groups on surfaces than the 15 kV nanofibers. Evidence that the 15 kV nanofibers had less -OH groups on surfaces is apparent when comparing the bulk behavior of crosslinked nanofibers. Regardless of GA concentration, 15 kV nanofibers were resistant to dissolution and film formation during sol-gel synthesis. The proposed mechanism of nanowrinkling was investigated by IR spectroscopy.
3.4.5. Spectroscopy of Nanofiber Surface Chemistry

FTIR absorbance spectra from 3000 to 3700 cm\(^{-1}\) gave insight into the quantity of free hydroxyl groups and their ability to engage in hydrogen bonding (Figure 3.7).\(^{120,124}\) Crosslinked nanofibers still had unreacted hydroxyl groups (Table 3.1). In some instances, the change in –OH concentration was negligible after crosslinking. It is also possible that water was absorbed during crosslinking with aqueous GA solution,\(^{122}\) as evidenced by fiber swelling or dissolution after crosslinking (Figure 3.3 and 3.4). The PVA -OH peak shifted to higher frequencies (from 3320 to 3376-3380 cm\(^{-1}\)) which indicates a reduction in hydrogen bonding after crosslinking 15 kV nanofibers. In contrast, intermolecular hydrogen bonding remained among the 45 kV nanofibers after crosslinking; -OH peaks ranged from 3310-3313 cm\(^{-1}\).

![FTIR Spectra](image)

**Figure 3.7.** FTIR spectra of PVA nanofibers that were electrospun at 15 or 45 kV before crosslinking with different GA concentrations.

The intensity of the acetal peak (C-O-C) increased after crosslinking relative to that of uncrosslinked nanofibers, as shown in Table 3.1. Acetal peak intensities were greater among
the 15 kV nanofibers than among the 45 kV nanofibers after crosslinking. Crosslinking with 400 mM GA increased the peak intensities for acetal bridge formation compared to 300 mM GA crosslinked fibers. Having fewer acetal peaks after crosslinking, free -OH groups among the 45 kV nanofibers were available to bond with Ti(OH)$_4$ during sol-gel synthesis.

Nanofiber crystallinity appears to have influenced TiO$_2$ nucleation and growth after crosslinking. Before crosslinking, 15 and 45 kV nanofibers had similar indices of crystallinity (see Table 3.1). After crosslinking, 15 and 45 kV electrospun nanofibers had shown increases in their crystallinity indices. Wang and Hsieh also reported crystallinity increases among electrospun PVA after GA crosslinking.$^{115}$ 15 kV nanofibers were reported to be more crystalline than the 45 kV nanofibers after crosslinking in 300 and 400 mM GA. Being less crystalline, 45 kV nanofibers were more susceptible to dissolution than the 15 kV nanofibers.
Table 3.1. Crystallinity and Acetal Bridge Formation of PVA Fibers Indicated by FTIR Spectra

<table>
<thead>
<tr>
<th>Sample ID* (Voltage, GA Molarity)</th>
<th>Hydroxyl groups ( \frac{A_{3320}}{A_{1450}} )</th>
<th>Acetal bridge ( \frac{A_{1097}}{A_{1450}} )</th>
<th>Crystallinity Index ( \frac{A_{1145}}{A_{854}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 kV, N/A</td>
<td>3.01</td>
<td>1.79</td>
<td>1.27</td>
</tr>
<tr>
<td>15 kV, 300 mM</td>
<td>2.99</td>
<td>2.91</td>
<td>5.67</td>
</tr>
<tr>
<td>15 kV, 400 mM</td>
<td>2.53</td>
<td>2.96</td>
<td>6.30</td>
</tr>
<tr>
<td>45 kV, N/A</td>
<td>2.81</td>
<td>2.09</td>
<td>1.63</td>
</tr>
<tr>
<td>45 kV, 300 mM</td>
<td>2.60</td>
<td>2.18</td>
<td>1.70</td>
</tr>
<tr>
<td>45 kV, 400 mM</td>
<td>3.02</td>
<td>3.47</td>
<td>5.87</td>
</tr>
</tbody>
</table>

* Sample ID describes the applied voltage for electrospinning and the molarity of GA within crosslinking solution.

Since the 45 kV nanofibers were presumed to have a greater concentration of -OH groups on the PVA fiber surfaces after crosslinking, XPS was used to investigate their surface chemistry (see Figure 3.8, Appendix B, Figure B4 & Table B2). The concentrations of -OH groups on the fiber surfaces were denser along the crosslinked 45 kV nanofibers than the crosslinked 15 kV nanofibers. The C 1s peak can represent binding energies of C=O (289 eV) or C-O-C (286.9 eV), C-O (286 eV), and C-C (284.8 eV). Interestingly, acetal C-O-C groups were only detected at the surface of 15kV, 300 mM GA nanofibers. The ratio of free surface hydroxyl groups (C-O) to crosslinked polymer (C-C + C-O-C) was 1.29 for 15 kV, 300 mM GA nanofibers. The ratio of C-O to C-C deconvoluted peak intensities was 0.8 for 15 kV, 400 mM GA nanofiber; 2.6 for 45 kV, 300 mM GA nanofiber; and 2.9 for 45 kV, 400 mM GA nanofiber. Detected C=O groups can come from PVA’s residual acetate groups or...
unreacted GA (Scheme 3.1a). By increasing the electromagnetic field strength (from 1.9 kV/cm – calculated by dividing the applied voltage of 15 kV by 8 cm of tip-to-collector distance - to 4.5 kV/cm – calculated by dividing the applied voltage of 45 kV by 10 cm of tip-to-collector distance -), the preferential migration of more PVA -OH groups towards the nanofiber surface was induced. PVA -OH groups within the bulk (opposed to the surface) were available for intermolecular crosslinking within nanofibers. This surface chemistry analysis provided evidence that TiO₂ nucleation was influenced by electrospinning voltage and PVA crosslinking degree.

Figure 3.8. XPS C 1s peaks of electrospun nanofibers designated as (a) 15 kV, 300 mM GA, (b) 45 kV, 300 mM GA, (c) 15 kV, 400 mM GA, and (d) 45 kV, 400 mM GA.
3.4.6. Swelling

Stress induced wrinkling among the surface-stiffened nanofibers was attributed to internal stresses. To investigate the internal stresses induced by swelling, the swelling of crosslinked PVA nanofibers in water (S_{water}) and IPA (S_{IPA}) were measured. According to Table 3.2, electrospinning voltage and GA concentration affected nanofiber swelling in both water and IPA. Among the 15kV, 300 mM GA nanofibers, water swelled nanofibers by 13% and IPA deswelled those nanofibers by 8%. Among the 45 kV, 300 mM GA nanofibers, 22 times more moisture swelling and 3 times more IPA deswelling than for the 15 kV, 300 mM GA nanofibers was measured.

Water swelling values correlated with changes in crystallinity index and the concentration of -OH groups on surfaces (Table 3.1 and Figure 3.8). 45 kV, 300 mM GA nanofibers- having the highest percentage of moisture swelling- were the least crystalline and most populated with -OH groups on the surfaces. Morton and Hearle suggested that non-crystalline polymer and the surface of crystalline polymer were most vulnerable to moisture absorption.\textsuperscript{39} Swelling behavior in IPA was affected by the degree of GA crosslinking as noted by acetylation and nanofiber moisture content. IPA is a nonsolvent for PVA; it densely packs crystalline polymer and removes residual water from PVA.\textsuperscript{131} The 45 kV, 300 mM GA nanofibers deswelled more in IPA than the 15 kV, 300 mM GA nanofibers. However, IPA swelling of the nanofibers increased with the molar concentration of GA crosslinker (Table 3.2).

Swelling data supported our hypothesis of each solvent’s role on wrinkle formation. The aggressive swelling and deswelling of 45 kV nanofibers (in water and IPA, respectively) caused differences in strain between their rigid outer layer and flexible core. Aligned
wrinkles then formed normal to the direction of radial swelling.\textsuperscript{33,110} Upon crosslinking and deswelling, the 45 kV, 400 mM GA nanofibers formed coarse wrinkles in IPA (Figure 3.4bi); whereas, TiO\textsubscript{2} sol-gel synthesis caused fine wrinkles along those nanofibers (Figure 3.4biv).

**Table 3.2.** Swelling of Electrospun PVA Nanofibers after 24 Hours of Water and IPA Immersion

<table>
<thead>
<tr>
<th>Sample name</th>
<th>S\textsubscript{water} (%)</th>
<th>S\textsubscript{IPA} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 kV, 300 mM</td>
<td>13</td>
<td>-8</td>
</tr>
<tr>
<td>15 kV, 400 mM</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>45 kV, 300 mM</td>
<td>291</td>
<td>-24</td>
</tr>
<tr>
<td>45 kV, 400 mM</td>
<td>39</td>
<td>2</td>
</tr>
</tbody>
</table>

Based on experimental data, Scheme 3.2 proposes a mechanism for understanding wrinkling along nanofibers having a crosslinked core. TTIP hydrolyzes to Ti(OH)\textsubscript{4}, which bonds with -OH groups of PVA fiber surfaces, and then Ti(OH)\textsubscript{4} condenses to TiO\textsubscript{2} nanoparticles. The nucleation of the stiff TiO\textsubscript{2} becomes the focal point of wrinkling.
Scheme 3.2. (a) Nanofiber surface chemistry and crosslinking by GA was influenced by electrospinning voltage. 45 kV nanofibers had more –OH groups on the surfaces than the 15 kV nanofibers. (b) Wrinkling along the 45 kV nanofibers resulted from TiO₂ sol-gel synthesis by means of cyclical swelling–deswelling. TiO₂ nucleation led to swelling-induced wrinkling.
3.5. Conclusions

A sol-gel approach to wrinkling nanofibers was introduced for the first time. Further, this technique advances the understanding of metal oxide nucleation and growth along interacting polymers. Based on these results, intermolecular bonding between sol-gel precursors and the polymeric surface were instrumental to mineralization and wrinkling. Having active metal oxide particles to align nanoscale grooves can spur the next generation of separation membranes and catalysts.

Herein, the role of surface chemistry and fiber microstructure on wrinkling was explored. Electrospinning was observed as a versatile technique for modifying the surface chemistry of polymers. By changing operating voltage, the preferential migration of electron donating groups was tuned under positive bias. Therefore, additives and polymer functionalization can be used to modify the nanofiber’s surface chemistry towards reaction. By spinning nanofibers at high voltage, a core-shell chemistry was induced, where hydroxyl groups populated the surface. In keeping with previous studies on wrinkling, having a rigid outer layer juxtaposed to a more expandable inner layer was key to the observation of wrinkles. Nanofiber spinning on the Elmarco Nanospider lends this technique to mass production for commercialization.
4.1. Abstract

Heavy metals are removed from water through chemical reactions, ion exchange or adsorption media, and bioremediation. Manganese, Mn(II), oxidizing fungi support bioremediation through the conversion of Mn(II) ions into manganese oxide (Mn(III/IV)O) deposits that in turn adsorb Mn(II) and other heavy metal ions from the environment. In this study, two fungal isolates, *Coniothyrium* sp. and *Coprinellus* sp., from a Superfund site (Lot 86, Farm Unit #1) water treatment system were incubated in the presence of nanofibers. Upon fungal attachment to manganese chloride seeded nanofibers, *Coniothyrium* sp.

---

3 The material in this chapter was submitted as: Park Y, Liu S, Gardner T, Ford E. Mycogenic Manganese Oxide Nanofibers for Heavy Metal Ions Removal. *Environmental Science and Technology.*
catalyzed the conformal deposition of Mn(III/IV)O along hyphae and nanofibers, but
*Coprinellus* sp. catalyzed Mn(III/IV)O only along its hyphae. Mn(III/IV)O coated nanofibers
(with and without *Coniothyrium* sp.) were most effective against (Mn(II)) and copper
(Cu(II)) metal ions removal than lead (Pb(II)).

**4.2. Introduction**

The release of heavy metal contaminants into groundwater poses a serious threat to
human and environmental health.62,63 Techniques used for heavy metal removal from water
include chemical precipitation reactions, ion exchange, adsorption, and bioremediation.132,64
Chemical precipitation is most widely used industrially, because the reactions are simple, and
its cost is relatively low. Heavy metal cations react with hydroxide or sulfide anions to form
water-insoluble salts. However, toxic by-products such as low-density sludge and hydrogen
sulfide (H₂S) fumes can persist upon reaction.64 Ion-exchange is a highly efficient, high
capacity heavy metal removal technique. Heavy metal ions attach to polymeric beads or
fibrous filter media as they replace protons belonging to sulfonic acid (-SO₃H) or carboxylic
acid (-COOH) groups on the surface.65

Metal carbonates, metal oxides, and carbon allotropes are commonly used inorganic
adsorbents. Heavy metal removal is influenced by the surface area of adsorbents. Thus,
nanoscale particles are expected to have greater adsorption efficiency per mass than
microscale adsorbents that are 100-10,000 times their size.63 Nanoparticle separation and
contamination is a major challenge to its use in water purification.66 Therefore, high surface
area nanofibers are presumed the best platforms for particle confinement and next-generation
hybrid materials.2
The electrospinning technique produces nanofibers from polymeric solutions that are placed under high voltage. Nanofibrous adsorbents are formed by electrospinning dopes containing nanosize particles or dopes containing sol-gel precursors for their synthesis. Also, inorganic particles are synthesized at the surface of nanofibers hydrothermally or by the sol-gel approach for particle synthesis along nanofibers. For example, Park et al. seeded electrospinning dopes with polymerized micelles to nucleate the hydrothermal growth of CaCO$_3$ spheres- which threaded the nanofibers. The CaCO$_3$ coated nanofibers adsorbed anthraquinone, acid blue dye from water. Wu et al. fabricated composite nanofibers of SiO$_2$ functionalized with thiol groups, that were capable of >400 mg/g of copper ion (Cu(II)) adsorption.

Bioremediation is most frequently used by the US Environmental Protection Agency (EPA) to treat groundwater in situ. Its popularity has grown since the 1990’s because the cost-benefit ratios for heavy metal and radionuclide removal by chemical treatments are deemed unacceptable. Bioremediation is a time-consuming strategy because it may take several months or years to identify the appropriate organisms and conditions for effective remediation. Nevertheless, the ubiquitous nature of microorganisms that can autonomously attack pollutants through oxidation and reduction makes bioremediation an attractive strategy for remediation long term.

Manganese, Mn(II), oxidizing bacteria and fungi are used to decontaminate polluted coal mine drainage sites. Limestone beds inoculated with Mn(II) oxidizing bacteria precipitate dark brown deposits of Mn(III/IV) oxide (i.e. Mn(III/IV)O), which adsorb other heavy metal ions from groundwater. Since Mn(II) oxidizing bacteria and fungi are found
throughout aquatic and terrestrial environments, biogenic Mn(III/IV)O naturally participate in environmental remediation.\textsuperscript{72,143,13,14}

\textit{Bacillus} sp. SG-1, \textit{Leptothrix discophora} SS-1, \textit{Pseudomonas putida} strains MnB1, and GB-1 are oxidizers of bacteriogenic Mn(III/IV)O.\textsuperscript{17,144} These bacteria secrete Mn(II) oxidative enzymes that catalyze the Mn(II) conversion to Mn(III/IV)O.\textsuperscript{17} Often times, the bacteria are found encrusted in Mn(III/IV)O deposits.\textsuperscript{17} Multicopper oxidase enzymes (like laccase and manganese peroxidase) have a similar effect, in that copper ions tend to contribute to the oxidization of various organic and inorganic substrates.\textsuperscript{17}

The formative and adsorptive behaviors of mycogenic Mn(III/IV)O are less understood than of bacteriogenic Mn(III/IV)O. Fungal activity is less susceptible to changes in pH, heavy metal ion concentrations, and temperature than bacteria.\textsuperscript{137} In practice, fungal Mn(II) oxidizers were more effective than bacterial Mn(II) oxidizers during the remediation of coal mine drainage.\textsuperscript{141} These ascomycetes were found to oxidize Mn(II)\textsuperscript{144} -Acremonium, \textit{Alternaria}, \textit{Cladosporium}, \textit{Coniothyrium}, \textit{Curvularia}, \textit{Penicillium}, \textit{Phoma}, \textit{Verticillium}-along with wood-rotting basidiomycetes (e.g. \textit{Coprinellus}).\textsuperscript{145} Fungi may also produce multicopper oxidases to aid Mn(II) oxidation,\textsuperscript{144} but the genetic sequence of multicopper oxidase from bacteria and fungi have little resemblance.\textsuperscript{17}

In general, biogenic Mn(III/IV)O have superior adsorption properties compared to synthetically produced Mn oxides. Unit cell forms of MnO$_6$ octahedra reassemble layered (e.g. birnessite) or tunneled structures (e.g. todorokite).\textsuperscript{143} Most biogenic birnessite contains a significant amount of Mn(III) in the octahedral layers.\textsuperscript{143} Mn(III/IV)O has an abundance of vacancies, which lends to its highly adsorptive nature.\textsuperscript{17,72} Dissolved salts are often found throughout the interstices of layered Mn(III/IV)O.\textsuperscript{143}
Biogenic Mn(III/IV)O\textsuperscript{146} and fungal cells\textsuperscript{147} are well-known bioadsorbents for heavy metals. Thus, hybrids of Mn(III/IV)O yielding fungi and nanofibers are posed as environmentally sound approaches for passive heavy metal removal from water. This novel approach immobilizes mycogenic adsorbents along the surface of nanofibers. The implications of this innovative approach are to support the scalable use of nanotechnologies towards groundwater treatment. Mn(III/IV)O morphologies, as catalyzed by \textit{Coprinellus} species (sp.) and \textit{Coniothyrium} sp., were investigated. The adsorptive capacity of \textit{Coniothyrium} sp. hybrids was tested against Mn(II), Cu(II), and lead (Pb(II)). The EPA secondary maximum contaminant levels for Mn(II) is 0.05 mg/L.\textsuperscript{148} EPA action levels are 1.3 mg of Cu(II)/L and 0.015 mg of Pb(II)/L in drinking water. If a tenth of tap water exceeds either action level, then additional treatments must be used to amend water systems.\textsuperscript{149}

4.3. Materials and Methods

4.3.1. Materials

Strains designated C1CAP-d1AYA and ASB2-d1KA, classified as \textit{Coprinellus} sp. and \textit{Coniothyrium} sp., respectively, were isolated from the activated carbon column (CC) and air-stripper (AS) of Superfund site, Lot 86, Farm Unit #1 remediation system in Raleigh, NC.\textsuperscript{150,151} Dextrose, yeast extract, casamino acids, HEPES acid, agar, CaCl\textsubscript{2}, MgSO\textsubscript{4}, MnCl\textsubscript{2}, FeCl\textsubscript{3}, CuSO\textsubscript{4}·5H\textsubscript{2}O, ZnSO\textsubscript{4}·7 H\textsubscript{2}O, CoCl\textsubscript{2}·6H\textsubscript{2}O, Na\textsubscript{2}MoO\textsubscript{4}·2H\textsubscript{2}O and 88\% hydrolyzed poly(vinyl alcohol) (PVA, having a molecular weight of \textasciitilde 130 kDa), 70\% glutaraldehyde (GA) in water, and 1 N sulfuric acid (H\textsubscript{2}SO\textsubscript{4}) were purchased from Sigma Aldrich.
4.3.2. Methods

4.3.2.1. Nanofiber Preparation

Neat PVA and PVA/MnCl₂ nanofibers were electrospun from aqueous dopes. 11 wt% of PVA was dissolved in 100 mL of water under constant stirring for 1 h at 60 °C. PVA/MnCl₂ was prepared from a 100 mL solution of 11 wt% PVA and 0.1 g of MnCl₂.

The laboratory scale electrospinning set-up is shown in Figure 4.2. PVA and PVA/MnCl₂ nanofibers were electrospun at 15 kV, horizontal tip to collector distance of 8 cm, and feed rate of 0.8 mL/h using an 18-gauge needle. Nanofibers were collected onto aluminum mesh at room temperature and 40-50% relative humidity.

![Figure 4.2. The schematic shows the laboratory-scale electrospinning set-up.](image)

4.3.2.2. Crosslinking Nanofibers

PVA and PVA/MnCl₂ nanofibers were crosslinked in GA solution. 300 mM GA in water/acetone was prepared from 70 % aqueous GA stock and 50 mL of acetone. One drop (~50 µL) of sulfuric acid catalyst was added. Nanofibers of 4 x 5 cm² were immersed into GA solution for 24 h at room temperature. Afterwards, nanofibers were air dried at room temperature for at least 24 h before use.
4.3.2.3. Media Preparation

Liquid Leptothrix media, with and without Mn(II) ions, were prepared in 3 steps. In Step (1), 1 g of dextrose, 0.5 g of yeast extract, 0.5 g of casamino acids and 2.38 g of HEPES acid were added to 1 L of distilled water. Afterwards in Step (2), this solution was autoclaved for 30 minutes and cooled to 60 °C using a room temperature water bath. Finally in Step (3), 0.5 mM of CaCl$_2$, 0.83 mM of MgSO$_4$, 1 mM of MnCl$_2$, 3.7 mM of FeCl$_3$, 1 mL of trace metal mix were added to prepare Leptothrix media having Mn(II) ions. Leptothrix media without Mn(II) did not have 1 mM of MnCl$_2$ added in Step 3.

For solid leptothrix media preparation, 15 g of agar was added in Step 1 with the same steps. Trace metal mix was prepared by adding 10 mg of CuSO$_4$·5H$_2$O, 44 mg of ZnSO$_4$·7H$_2$O, 20 mg of CoCl$_2$·6H$_2$O, and 13 mg of Na$_2$MoO$_4$·2H$_2$O in solution. Afterwards, the trace metal mix was filtered through sterile 0.2 µm porous filters.

4.3.2.4. Fungal treatment of Nanofibers

Leptothrix culture media was inoculated with *Coprinellus* sp. and *Coniothyrium* sp. in the presence of nanofibrous sheets (of 1x1 cm$^2$ or 4x5 cm$^2$). Nanofibers and fungal plugs were simultaneously added to liquid media. On solid plates, fungal plugs were placed on top of nanofibers, under sterile conditions. PVA and PVA/MnCl$_2$ nanofibers were immersed in 20-30 mL of Leptothrix culture media in 75 mL Erlenmeyer flasks. Fungal growth over 4 weeks occurred at room temperature in the dark.

The fungi were removed from the fungi-nanofiber hybrids by immersion in 20 mL of deionized water, stirring at 500 rpm for 30 min, or sonication in 20 mL of water or ethanol for 2 h. Table 4.1 summarizes the samples used throughout this study and their descriptions.
Table 4.1. Summary of Nanofibers with and without Fungal Treatment

<table>
<thead>
<tr>
<th>Sample Name (Optional)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVA</td>
<td>Neat electrospun PVA nanofibers</td>
</tr>
<tr>
<td>PVA/MnCl(_2)</td>
<td>PVA nanofibers having 0.1 wt% MnCl(_2)</td>
</tr>
<tr>
<td>Mn(III/IV)O-#17</td>
<td><em>Coniothyrium</em> sp. incubated in Mn(II)-containing media and have Mn(III/IV)O</td>
</tr>
<tr>
<td>-</td>
<td><em>Coniothyrium</em> sp. incubated in Mn(II)-containing media with PVA nanofibers</td>
</tr>
<tr>
<td>Mn(III/IV)O-#17-PVA/MnCl(_2)</td>
<td><em>Coniothyrium</em> sp. incubated in Mn(II)-containing media with PVA/MnCl(_2) nanofibers</td>
</tr>
<tr>
<td>Mn(III/IV)O-PVA/MnCl(_2) (-#17)</td>
<td>Mn(III/IV)O-PVA/MnCl(_2) after removing <em>Coniothyrium</em> sp. hyphae with 2h of ethanol sonication</td>
</tr>
<tr>
<td>-</td>
<td>Mn(III/IV)O-PVA after removing <em>Coprinellus</em> sp. hyphae with 2h of ethanol sonication</td>
</tr>
<tr>
<td>-</td>
<td>Mn(III/IV)O-PVA/MnCl(_2) after removing <em>Coprinellus</em> sp. hyphae with 2h of ethanol sonication</td>
</tr>
</tbody>
</table>

4.3.2.5. Microscopic Analysis

Confocal micrographs of fungi incubated on Leptothrix solid plates and in the presence of nanofibers were imaged on the LEXT OSL4000 3D. The morphology of fungi-nanofibers and nanofibers were observed using field emission scanning electron microscopy (FE-SEM, FEI, Verios 460L) at an operating voltage of 2 kV. Specimens were sputter coated with gold and palladium before SEM analysis. Image analysis of SEM micrographs was used to calculate the average fiber diameter from at least 50 nanofibers.

4.3.2.6. Spectroscopic Analysis of Mn(III/IV)O

The surface chemistry of nanofibers and their mycogenic hybrids were characterized by energy dispersive spectroscopy (EDS, attached to the Verios 460L). The operating voltage
was 20 kV. EDS spectra were used to confirm the locale of metal oxides and absorbed heavy metals.

The Nomadic™ Raman Microscope by BaySpec was used to analyze Mn(III/IV)O nanofibers. Raman spectra were collected using the 532 nm laser at 150 mW power, 10X objective lens, 20 scans, and 1 sec exposure time.

4.3.2.7. Heavy Metal Adsorption

Heavy metal sorption by PVA/MnCl₂, Mn(III/IV)O-#17, Mn(III/IV)O-#17-PVA/MnCl₂, and Mn(III/IV)O-PVA/MnCl₂(-#17) were measured. 3 mg of each sample was added to 20 mL of the metal ion solution.

Specimen were immersed in heavy metal solutions (of 0.55 mg Mn(II)/L, 2.63 mg Cu(II)/L, and 0.029 mg of Pb(II)/L) at pH 7 for 24 h at room temperature. Inductively-Coupled Plasma-Optical Emission Spectrometer (ICP-OES, Perkin Elmer 8000) was used to measure unabsorbed concentrations of heavy metal ions. Adsorption \( q \) (mg/L) was determined by Equation 4.1:

\[
q = V \frac{(C_i - C)}{S} \quad (4.1)
\]

\( V \) is the volume (L) of solution in contact with sorbent; \( C_i \) and \( C \) are initial and final concentrations of the sorbate (mg/L), respectively; \( S \) is the dry weight of sorbent (g). Heavy metal removal (%) was determined by Equation 4.2:

\[
Removal \( (%) \) = \frac{(C_i - C)}{C_i} \times 100 \quad (4.2)
\]

4.4. Results and Discussion

4.4.1. Fungal Growth and Mn(III/IV)O Deposition

Photographs of fungi grown in Leptothrix media revealed white, cotton ball-like mycelia (Figure 4.3a,b). Dark brown deposits, indicative of Mn(III/IV)O,\(^{13,152} \) formed after 1 week of
*Coprinellus* sp. and *Coniothyrium* sp. incubation in Mn(II)-containing media (Figure 4.3a,b). Adding PVA/MnCl₂ nanofibers into media without Mn(II) did not yield the Mn(III/IV)O deposits (Figure 4.3a,b). The average diameter of PVA nanofibers without MnCl₂ was 186 ± 116 nm, and PVA/MnCl₂ nanofibers was 126 ± 46 nm (Figure 4.3c,d respectively).

Interestingly, PVA/MnCl₂ nanofibers yielded more mycelia growth, and hyphal structures tended to adhere more strongly to nanofibers in comparison to neat PVA nanofibers that were immersed in Mn(II)-containing media (Figure 4.3a,b). Likewise, more mycelia and Mn(III/IV)O grew on PVA/MnCl₂ nanofibers when grown on Leptothrix solid plates (Appendix C, Figure C1). Confocal microscopy images (Appendix C, Figure C2) showed Mn(III/IV)O deposits only on PVA/MnCl₂ fibers cultured in Mn(II)-containing media.

Fungal growth in the presence of nanofibers and Mn(II) was monitored over 4 weeks (Figure 4.3e). Incubation beyond 2 weeks did not enhance the spread of Mn(III/IV)O deposits along nanofibers. Nevertheless, nanofibers were maintained in culture media for more than 4 weeks to obtain fully grown fungi.¹³ PVA/MnCl₂ nanofibers were more densely covered with mycelia. Mn(II) ions within nanofibers were assumed to promote fungal growth and Mn(III/IV)O formation. While studying the effect of manganese ions at varying concentrations on select fungi isolates, Jaworska et al. reported that the fungal growth of *Trichoderma spp.* to manganese ions was affected by both the metal concentration and the fungal isolate.¹⁵³
Figure 4.3. Fungi (a) *Coprinellus* sp. and (b) *Coniothyrium* sp. were grown for 1 week in Leptothrix media with Mn(II) or without Mn(II) (i.e. -Mn(II)). Fungi (a and b) were incubated without nanofibers (of 1x1 cm$^2$) or with nanofibers. Nanofibers were either of neat PVA or PVA/MnCl$_2$. SEM images of (c) PVA and (d) MnCl$_2$/PVA nanofibers are shown. Photographs show (e) *Coniothyrium* sp. grown for up to 4 weeks in Mn(II)-containing media 4x5 cm$^2$ sheets of nanofiber (either PVA or PVA/MnCl$_2$). This growth pattern was also observed for *Coprinellus* sp.

The attachment of *Coprinellus* sp. and *Coniothyrium* sp. mycelia to nanofibers was confirmed by inspection of SEM images (Figure 4.4). Mn(III/IV)O morphology can differ between each fungal species.$^{13}$ *Coprinellus* sp. catalyzed the growth of spherical
Mn(III/IV)O particles when cultured in Mn(II)-containing media. Spherical Mn(III/IV)O particles were more prominent along the hyphae of PVA/MnCl₂ nanofibers than on neat PVA nanofibers. *Coniothyrium* sp. catalyzed the growth of sheet-like Mn(III/IV)O in Mn(II)-containing media. Extracellular matrix appeared at the interface of nanofibers and *Coniothyrium* sp. hyphae. Round spores were observed as seen in Figure 4.4bi. Fruiting bodies were also visible in photographs (Appendix C, Figure C2). Thus, nanofibers did not inhibit fungal reproduction by spores.
Figure 4.4. Micrographs show (a) *Coprinellus* sp. (b) *Coniothyrium* sp. grown on PVA and PVA/MnCl$_2$ nanofibers immersed in -Mn(II) or +Mn(II) liquid media.
4.4.2. Elemental Analysis of Fungal Mn(III/IV)O Hybrids

Mn(II) within PVA/MnCl₂ fibers were not detected by EDS, because Mn(II) at the surface of PVA/MnCl₂ nanofibers was below the EDS detection limit, i.e. 0.1 wt%. Therefore, any manganese detection must result from the oxidation of Mn(II) in solution. With both fungal types, manganese content as measured by EDS was qualitatively higher along PVA/MnCl₂ than neat PVA nanofibers (Figure 4.5). EDS spectra showed impurities of Fe, S, Mg, and Ca within Mn(III/IV)O. As expected, Mn(III/IV)O can adsorb other metal ions from Leptothrix media. Ivarsson et al. detected non-Mn(II), metal ions within the vacancies of biogenic Mn(III/IV)O by EDS.¹⁵² Coniothyrium sp. can release enzymes coupled with P, S, K, and Ca.
Figure 4.5. EDS graphs of *Coprinellus* sp. (a) *Coniothyrium* sp. (b) on PVA (i, iii) or PVA/MnCl$_2$ nanofibers (ii, iv) in liquid media -Mn(II) and with +Mn(II).
4.4.3. Mn(III/IV)O Deposition on Nanofibers

A layer of fungal hyphae intermeshed with nanofibers is illustrated in Figure 4.6: Mn(III/IV)O-#17-PVA/MnCl₂. Cracks formed in the topical layer of Coniothyrium sp. after stirring hybrids immersed in water (Figure 4.6a). The cross-sectional view shows hyphae attached to both sides of nanofibers in a sandwich structure (Figure 4.6b). After sonicating the sample for 1 h in water, some fungal hyphae remained on the nanofibers (Figure 4.6c). But sonicating the fungi-nanofiber hybrids for 2 h in ethanol removed most of the fungal hyphae (Figure 4.6d). This latter technique was used to remove fungus from hybrids that were incubated in Mn(II)-containing media.

EDS was used to investigate the deposition of Mn(III/IV)O onto nanofibers as a result of their adherence to Mn(II) oxidizing fungus. Mn was not detected on the surface of PVA and PVA/MnCl₂ nanofibers that were incubated with Coprinellus sp. (Figure 4.7a,b). Also, Mn was not identified on the surface of neat PVA nanofibers that were incubated with Coniothyrium sp. (Figure 4.7c). Mn(III/IV)O coating on PVA/MnCl₂ nanofibers, as catalyzed by Coniothyrium sp., was durable to withstand 2 h of sonication in water. Mn was detected on the surface of Mn(III/IV)O-PVA/MnCl₂(-#17) nanofibers. A conformal Mn(III/IV)O coating was found on the fiber surface after 2 h of ethanol sonication (Figure 4.7d). Figure 4.8 is an EDS chemical map of manganese, oxygen, and carbon distributions along mineralized nanofibers.
Figure 4.6. SEM images of Mn(III/IV)O-Coniothyrium sp.-PVA/MnCl₂ after water immersion and magnetic stirring for 30 min at 500 rpm; both (a) longitudinal and (b) cross sectional views are shown. Hybrids were further treated with (c) 1 h of sonication in water or (d) 2 h of sonication in ethanol.
Figure 4.7. (i) SEM images and (ii) EDS spectra from *Coprinellus* sp. incubated with (a) PVA and (b) PVA/MnCl$_2$ nanofibers are shown. (i) SEM images and (ii) EDS spectra of *Coniothyrium* sp. incubated with (c) PVA and (d) PVA/MnCl$_2$ nanofibers are shown.
Figure 4.8. EDS map of Mn(III/IV)O-PVA/MnCl₂ (#17) shows C in red, Mn in green, and O in blue.

Fungi can secrete various oxidative enzymes as their hyphae continue to lengthen. This results in the cycling of nutrients throughout the environment. Manganese oxidizing fungi express extracellular oxidative enzymes such as manganese peroxidase. This group of proteins are capable of oxidizing Mn(II) to Mn(IV) at accelerated rates of Mn biomineralization, i.e., several orders of magnitude faster than either abiotic catalysis on mineral surfaces or homogeneous oxygenation in aqueous solution. Coprinellus sp. and Coniothyrium sp. apparently have different secretion modes of Mn(II) oxidizing enzymes. Manganese-oxidizing enzymes were mostly associated with the hyphae cells of Coprinellus sp. In contrast, enzymes were secreted from Coniothyrium sp. hyphae. Oxidizing enzymes were captured by the extracellular matrix which exuded from Coniothyrium sp. and onto the surrounding nanofibers (Figure 4.4b). Santelli et al. reported that locale of Mn(III/IV)O
deposition (i.e. on hyphal surfaces or extracellular polymer adjacent to cells) varies among different Ascomycete fungi probably due to primary placement of enzymes.\textsuperscript{13}

Raman spectra in Figure 4.9 show characteristic bands of Mn(III/IV)O\textsuperscript{152} in the range of 480-700 cm\textsuperscript{-1} for Mn(III/IV)O-PVA/MnCl\textsubscript{2}(-#17). Low Raman intensities were observed for Mn(III/IV)O, which resembled birnessite, as reported by Julien et al.\textsuperscript{155} Yu et al. reported Mn(III/IV)O catalyzed by \textit{Paraconiothyrium} sp. (a \textit{Coniothyrium}-like ascomycete) is birnessite.\textsuperscript{156} The layered birnessite structure can adsorb metal ions between atomic layers and vacancies found throughout its imperfect crystal structure.\textsuperscript{17} In the next section, heavy metal adsorption by \textit{Coniothyrium} sp. derived Mn(III/IV)O hybrids were tested.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4_9.png}
\caption{Raman spectra of nanofibers treated with \textit{Coprinellus} sp. and \textit{Coniothyrium} sp.}
\end{figure}
4.4.4. Heavy Metal Adsorption by Coniothyrium sp. Hybrids

Mn(III/IV)O- Coniothyrium sp. had shown the best properties of heavy metal sorption per mass of sorbent (Table 4.2). Hybrid nanofibers removed heavy metals from solution in order from Mn(II) > Cu(II) >> Pb(II). The molecular size of heavy metal ions may have been size excluded from vacancies within the Mn(III/IV)O coating. Laus and Favere also associated smaller radius of Cu(II) than that of Cd(II) to better adsorption by chitosan. After adsorption experiments, the surface chemistry of PVA/MnCl₂ and Mn(III/IV)O-PVA/MnCl₂(-#17) nanofibers were mapped by EDS. Figure 4.10 shows the distribution of Cu(II) and Mn(II) by both types of nanofibers. As expected from Table 4.2 results, Cu(II) detected along Mn(III/IV)O coated nanofibers was more intense than along PVA/MnCl₂.

Both Mn(III/IV)O-#17-PVA/MnCl₂ and Mn(III/IV)O-PVA/MnCl₂(-#17) removed more than 90% of the Mn(II) from the water. The nanoscale dimensions of Mn(III/IV)O coated nanofibers appear to have enhanced Mn(II) sorption capacities. Taffarel and Rubio reported 4 g/L of manganese oxide coated zeolite achieved 90% removal of Mn(II) from the water after 2 h, whereas Mn(II) was more efficiently removed from water by 150 mg/L of Coniothyrium sp.-nanofiber derivatives Mn(II). EXAFS spectra examinations reported by Duckworth et al., also indicate that mycogenic manganese oxides from Lot 86 biofilm containing these particular isolates are similar in both the structure of Mn(III/IV)O and the binding of Ba, Co, and Zn to those observed with laboratory-grown bacteriogenic Mn(III/IV)O.

Pb(II) has an atomic radius that is significantly larger than Mn(II) and Cu(II). Thus, its size can hinder Pb(II) adsorption into the vacancies of Mn(III/IV)O and its coordination with atoms on the surfaces through nonspecific bonding. Nelson et al. reported Pb(II) adsorption
by bacteriogenic Mn(III/IV)O and synthetic β-MnO₂ were 1344.67 mg/g and 0.078 mg/g, respectively.¹⁵⁹ Droz et al. noted that bacteriogenic Mn(III/IV)O (from Pseudoonas putida GB-1) had more structural defects than mycogenic Mn(III/IV)O (from Coprinellus sp.).¹⁴⁵

Table 4.2. Comparing Aqueous Heavy Metal Adsorption by Coniothyrium sp. Derived Mn(III/IV)O Coatings and Nanofibers After 24 h

<table>
<thead>
<tr>
<th></th>
<th>Mn(III/IV)O-#17</th>
<th>PVA/MnCl₂</th>
<th>Mn(III/IV)O-#17-PVA/MnCl₂</th>
<th>Mn(III/IV)O-PVA/MnCl₂(-#17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn(II) Removal (%)</td>
<td>67 ± 0</td>
<td>18 ± 0</td>
<td>93 ± 0</td>
<td>99 ± 0*</td>
</tr>
<tr>
<td>Mn(II) adsorption (mg/g)</td>
<td>2.69 ± 0.01</td>
<td>0.66 ± 0.01</td>
<td>3.41 ± 0.00</td>
<td>3.65 ± 0.00*</td>
</tr>
<tr>
<td>Cu(II) Removal (%)</td>
<td>58 ± 0</td>
<td>38 ± 0</td>
<td>66 ± 0</td>
<td>57 ± 0</td>
</tr>
<tr>
<td>Cu(II) adsorption (mg/g)</td>
<td>10.22 ± 0.08</td>
<td>6.62 ± 0.04</td>
<td>11.64 ± 0.03</td>
<td>10.04 ± 0.03</td>
</tr>
<tr>
<td>Pb(II) Removal (%)</td>
<td>52 ± 8</td>
<td>43 ± 4</td>
<td>30 ± 5</td>
<td>18 ± 5</td>
</tr>
<tr>
<td>Pb(II) adsorption (mg/g)</td>
<td>0.10 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.04 ± 0.01</td>
</tr>
</tbody>
</table>

* Mn(II) concentration after Mn(III/IV)O-PVA/MnCl₂ immersion was less than the detection limit of 0.005 mg/L.

** Original concentrations of each heavy metal solutions were: 0.55 mg/L Mn(II), 2.63 mg/L Cu(II), and 0.029 mg/L Pb(II) at pH 7 as measured by ICP-OES.

87
Figure 4.10. EDS chemical mapping of (a) PVA/MnCl$_2$ and (b) Mn(III/IV)O-PVA/MnCl$_2$-(#17) nanofibers after Cu(II) adsorption: (i) C in red, (ii) O in blue, (iii) Cu in green, and (iv) Mn(II) in cyan.
4.5. Conclusions

Two different phyla of indigenous manganese oxidizing fungi assemblages collected from the polluted groundwater under remediation at Lot 86 were grown on nanofiber scaffolds to combine the best practices of bioremediation and nanotechnology. The high surface area of nanofibers provided excellent substrates for filamentous hyphae that are on the order of micrometers. *Coprinellus* sp. and *Coniothyrium* sp. are plant pathogens, but neither of them poses known threats to human health. *Coniothyrium* sp. catalyzed the conformal deposition of Mn(III/IV)O along the nanofibers unlike *Coprinellus* sp., which oxidized Mn(II) along its hyphae. Our results showed that *Coniothyrium* sp. and *Coprinellus* sp. have different mechanisms of exuding oxidative enzymes. In turn, each yielded different forms of Mn(III/IV)O particles.

Oxidative enzymes released from *Coniothyrium* sp. migrated to PVA/MnCl$_2$ nanofibers, as Mn(II) diffused to the surface of water-swellable nanofibers. In turn, Mn(III/IV)O formed a conformal coating along nanofibers. Herein, a novel approach to mineralization was observed- where oxidative enzymes external to the nanofibers catalyzed the nucleation and growth of Mn(III/IV)O along the nanofibers when Mn(II) was in the media. For synthetic mineralization, nanofibers are typically seeded with polyelectrolytes to nucleate the growth of inorganic minerals hydrothermally, as observed in Park et al$^{113}$ and Yang et al$^{23}$ work on CaCO$_3$ nanofibers. Having the nanofibers scaffold Mn(III/IV)O mitigates contamination from unconfined fungus and inorganic particulates in the water.

It is noteworthy that hyphae-nanofiber derivatives removed heavy metals as well if not better than Mn(III/IV)O coated hyphae alone. Mn(III/IV)O coated nanofibers effectively reduced Mn(II) contamination by 90 times- which is well below the EPA limit- in 24 hours.
CHAPTER 5
SUMMARY & CONCLUSIONS

Novel techniques for nanofiber mineralization mimicked biological approaches. The natural role of proteins in bone formation inspired research on CaCO$_3$ mineralized nanofibers, and the natural occurrence of wrinkling aided our understanding of how TiO$_2$ sol-gel synthesis induces nanowrinkles along nanofibers. Further, manganese oxidizing fungi and oxidative enzymes were used as biocatalysts for Mn(III/IV)O formation. Both CaCO$_3$-nanofibers and Mn(III/IV)O-nanofibers were tested against water contaminants (anthraquinone dye and heavy metals) removal. The scaffolding of functional, inorganic particles along nanofibers has several advantages for water treatment:

1) high surface area, nanoscale materials make idea adsorbents
2) confining the nano- to microscale adsorbents mitigates secondary contamination by nanoparticles.
3) microstructure of inorganic sorbents and their performance can be controlled by surface chemistry of nanofibers

The surface chemistry of nanofibers affected the nucleation and growth of inorganic coatings. From this study, we have suggested two approaches for tuning the surface chemistry of electrospun nanofibers:

1) seeding spinning dopes with polymerized micelles or other electrolytes
2) control applied voltage during electrospinning to tune the dielectrophoretic response and migration of molecular functional groups towards the nanofiber surface.

In chapter 2, proteins that nucleate and grow hydroxyapatite crystals along collagen fibril in bone formation were emulated with polymerized micelles. Polymerized micelles migrated
to the nanofiber surface during electrospinning and helped to nucleate Ca$^{2+}$ and CO$_3^{2-}$ ions during CaCO$_3$ mineralization. The presence of these micelles resulted in the highest degrees of CaCO$_3$ mineralization by the alternating dipping method. Additionally, nanofibers seeded with micelles yielded vaterite CaCO$_3$, which enhance contaminant adsorption.$^{79}$

We recommend further comparison of the process-structure-property relationships that exist for electrospinning on laboratory and pilot scales. Nanofibers were spun at 15kV on the custom-built electrospinning machine, and nanofibers were spun at 45 kV on the pilot scale Elmarco Nanospider Elmarco. The wrinkling study revealed differences in surface chemistry as the result of voltage potential. Thus, more research is needed to understand the effects of voltage potential on nanofiber properties. Such efforts could lead to unique properties that are only observed on commercial equipment and lead to the commercialization of unique nanofibers.

In chapter 3, the novel formation of aligned nanowrinkles were observed in consequence to the sol-gel synthesis of TiO$_2$ nanoparticles along electrospun nanofibers. To our knowledge, this is the first time that these structures have been reported. By electrospinning nanofibers at high positive voltage (45 kV), more hydroxyl groups populated the nanofiber surface than at low voltage (15 kV). This difference in electrospinning potential affected PVA crystallinity among the electrospun nanofibers. After crosslinking, 45 kV nanofibers underwent more extreme swelling in water and deswelling in IPA solutions for TiO$_2$ sol-gel synthesis. Having a rigid outer layer juxtaposed to a more extensible inner layer of 45 kV fibers was key to the aligned wrinkle formation.

The TiO$_2$ that formed along wrinkled nanofibers was non-crystalline. This form was not capable of catalyzing the photocatalytic degradation of organic molecules. Nonetheless, the
adsorbent properties of amorphous TiO$_2$ is cause for further investigation. Wrinkled nanofibers having non-crystalline TiO$_2$ may therefore find use in water treatment. The pattern of aligned wrinkles is expected to assist with chemical separation.

In chapter 4, manganese oxidizing fungi were grown on nanofibers. *Coniothyrium* sp. catalyzed the conformal deposition of manganese oxides along the surface of nanofibers seeded with Mn(II). We propose *Coniothyrium* sp. had exuded oxidative enzymes onto the surface of nanofibers, which in turn oxidized Mn(II) to Mn(II/IV)O deposits. High surface area of nanofibers provided excellent substrates for microscale, filamentous hyphae and manganese oxides. The nanofibrous scaffolds helped to prevent secondary contamination of water, as caused by the leaching of hyphae and inorganic particles. It is noteworthy that manganese oxide coated nanofibers had similar (Pb$^{2+}$, Cu$^{2+}$, Mn$^{2+}$) heavy removal properties as hyphae-nanofiber composites.

Since the oxidative enzymes that exuded from fungi could form manganese oxide deposits along nanofibers, we recommend the seeding of nanofibers with oxidative enzymes. Upon seeding, we believe these nanofibers could form manganese oxide deposits along the nanofibers. This will provide sustainable textile finishing with desired functions such as water filtration and energy storage.
APPENDIX A. PROPERTIES OF SURFMER SOLUTIONS

Figure A1. Plots of conductivity and surface tension measurements were used to estimate surfmer CMC.

Figure A2. Particle size distributions for unreacted PAMS and PAPS surfmers at 0.01, 0.1, and 1 wt% were measured by DLS.
APPENDIX B

FIBER PROPERTIES AFTER TiO₂ INDUCED WRINKLING

<table>
<thead>
<tr>
<th>Before Crosslinking</th>
<th>Alternative dipping 0</th>
<th>1</th>
<th>3</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 kV 300 mM 15 kV</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>45 kV 300 mM 45 kV</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>15 kV 400 mM 15 kV</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>45 kV 400 mM 45 kV</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
</tbody>
</table>

**Figure B1.** Photograph of PVA nanofibers before and after TiO₂ sol-gel synthesis by the cyclical dipping method.

As observed in Figure B1, the appearance of poly(vinyl alcohol), PVA, nanofibers after crosslinking and TiO₂ sol-gel synthesis was influenced by applied voltage. The 15 kV nanofibers were opaque and remained flexible after TiO₂ synthesis. In contrast, the 45 kV nanofibers were translucent and became stiffer after TiO₂ synthesis.
Nanofiber preparation and sol-gel dipping cycles affected sol-gel induced morphologies. Wrinkled morphologies shown in Figure B2 have their wavelengths determined in Table B1.

**Figure B2.** Scanning electron microscopy (SEM) of electrospun nanofibers after sol-gel induced wrinkling.

**Table B1.** Average Wrinkling Wavelength as Measured from SEM Images of Wrinkled 45 kV Nanofibers.

<table>
<thead>
<tr>
<th></th>
<th>45 kV, 300 mM</th>
<th>45 kV, 300 mM</th>
<th>45 kV, 400 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 TiO₂ Sol-Gel Cycles</td>
<td>115 ± 22 nm</td>
<td>231 ± 42 nm</td>
<td>191 ± 32 nm</td>
</tr>
<tr>
<td>5 TiO₂ Sol-Gel Cycles</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure B3. SEM images show (a) 15 kV nanofibers that were crosslinked with 300 mM GA and treated with 5 alternating dipping cycles of IPA and water. 45 kV nanofibers are shown after crosslinking in 300 mM GA, (b) 1, (c) 3, and (d) 5 cycles of IPA and water dipping.

The effect of solvent- from sol-gel synthesis- on nanofiber morphology was studied. Titanium isopropoxide (TTIP) was dissolved in isopropanol (IPA). Water was used as a wetting and rinsing solvent. Nanofibers were electrospun at 15 and 45 kV prior to crosslinking in 300 mM GA and undergoing up to 5 cycles of dipping in IPA and water. Wrinkling was not observed along 15 kV and 45 kV nanofibers, in spite of IPA and water dipping (Figure B3). The structure of 15 kV nanofibers remained after 5 cycles of IPA and water dipping, but the structures of 45 kV nanofibers were mostly replaced by film. Film formation implied PVA dissolution. The translucence of 45 kV nanofibers after IPA/water dipping also implied losses in PVA crystallinity.
X-ray photoelectron spectroscopy, XPS of O 1s and C 1s binding energies, were used to differentiate between the surface chemistries of crosslinked PVA nanofibers that were spun at 15 and 45 kV (Figure B4). The atomic composition of those nanofibers after crosslinking are shown in Table B2.

![Overall XPS spectra of nanofibers electrospun at 15 and 45 kV are shown. PVA nanofibers were crosslinked with (a) 300 or (b) 400 mM GA.]

**Figure B4.** Overall XPS spectra of nanofibers electrospun at 15 and 45 kV are shown. PVA nanofibers were crosslinked with (a) 300 or (b) 400 mM GA.

**Table B2.** Atomic Composition of Crosslinked PVA Nanofibers by XPS

<table>
<thead>
<tr>
<th>Atomic Composition (%)</th>
<th>C 1s</th>
<th>O 1s</th>
<th>O/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 kV, 300 mM</td>
<td>70.1</td>
<td>29.9</td>
<td>0.4</td>
</tr>
<tr>
<td>45 kV, 300 mM</td>
<td>66.8</td>
<td>26.3</td>
<td>0.4</td>
</tr>
<tr>
<td>15 kV, 400 mM</td>
<td>67.6</td>
<td>26.7</td>
<td>0.4</td>
</tr>
<tr>
<td>45 kV, 400 mM</td>
<td>61.5</td>
<td>30.9</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Figure C1. *Coprinellus* sp. (a) and *Coniothyrium* sp. (b) were grown in the dark at room temperature for 1 week on solid plates without Mn(II) (-Mn(II)) and with Mn(II) (+Mn(II)). Neat PVA nanofibers or PVA nanofibers containing MnCl₂ were added into leptothrix solid media.
Figure C2. Confocal microscope images of (a) *Coprinellus* sp. and *Coniothyrium* sp. on solid plates, (b) *Coprinellus* sp. and (c) *Coniothyrium* sp. grown on PVA or PVA/MnCl$_2$ fibers in liquid media with and without Mn(II).
REFERENCES


(15) Bansal, V.; Rautaray, D.; Bharde, A.; Ahire, K.; Sanyal, A.; Ahmad, A.; Sastry, M.


(22) Liu, L.; He, D.; Wang, G. S.; Yu, S. H. Bioinspired Crystallization of CaCO3 Coatings


(40) Wang, X.; Ge, J.; Si, Y.; Ding, B. Adsorbents Based on Electrospun Nanofibers; Ding, B., Yu, J., Eds.; Nanostructure Science and Technology; Springer Berlin Heidelberg: Berlin, Heidelberg, 2014; pp 473–495.


(45) Yener, F.; Jirsak, O. Comparison between the Needle and Roller Electrospinning of


(59) Ford, E. N. J.; Suthiwangcharoen, N.; D’Angelo, P. A.; Nagarajan, R. Role of Single-Walled Carbon Nanotubes on Ester Hydrolysis and Topography of Electrospun Bovine


(75) Nasreen, S. A. A. N.; Sundarrajan, S.; Nizar, S. A. S.; Balamurugan, R.; Ramakrishna, S. Advancement in Electrospun Nanofibrous Membranes Modification and Their


(82) Baláž, M.; Bujňáková, Z.; Baláž, P.; Zorkovská, A.; Danková, Z.; Briančin, J. Adsorption of cadmium(II) on Waste Biomaterial. *J. Colloid Interface Sci.* 2015, 454,


(91) Braganza-pugh, S. Role of Reactive Surfactants in Miniemulsion Polymerization, Lehigh University, 2010.


Datla, V. M. Surface Modification of Fibers and Nonwovens with Melt Additives, North Carolina State University, 2008.


2010, 9 (2), 159–164.


(127) Fu, Q.; Jin, Y.; Song, X.; Gao, J.; Han, X.; Jiang, X.; Zhao, Q.; Yu, D. Size-Dependent Mechanical Properties of PVA Nanofibers Reduced via Air Plasma Treatment. 


(148) Environmental Protection Agency (EPA). Secondary Drinking Water Standards: Guidance for Nuisance Chemicals


