

Bioengineered fibers for localized and sustained drug delivery

by

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B.S., Worcester Polytechnic Institute, 2006

Submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy
in the Program of Biomedical Engineering at Brown University

Providence, Rhode Island

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This dissertation by Danya M. Lavin is accepted in its present form by the program of
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for the degree of Doctor of Philosophy.

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EDUCATION

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| 2012 | Ph.D., Biomedical Engineering
Brown University, Providence, RI |
| 2006 | B.S., Biomedical Engineering, <i>high distinction</i>
Minor, German Language
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RESEARCH EXPERIENCE

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| 2006 – | Graduate Research Assistant , Department of Molecular Pharmacology, Physiology, and Biotechnology, Brown University, Providence, RI
<i>Bioengineered Fibers for Localized and Sustained Drug Delivery</i>
Mentors: Professor Edith Mathiowitz and Dr. Richard A. Hopkins <ul style="list-style-type: none">• Developed wet solution spinning methodologies for fabricating fiber-based drug delivery systems• Encapsulated therapeutics and characterized release kinetics, biological activity, and intrinsic material properties of delivery systems• Designed and implemented surgical experiments using a juvenile sheep animal model towards the development of a drug delivery suture• Consulted on various research/industry projects investigating growth factor/drug delivery and tumor metastasis in the areas of cartilage regeneration, treatment of IRBD, and screening of novel chemotherapeutics |
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(Research Experience – continued)

2005 – 2006

Senior Undergraduate Major Qualifying Project (MQP),
Department of Biomedical Engineering, WPI, Worcester, MA
Comparison of Cancellous and Cortical Bone Screws for Sternal Application

Mentor: Professor Kristen L. Billiar

- Developed a methodology for testing repetitive cyclic loading of low loads on porcine sterna to investigate the effects of repetitive cyclic fatigue on the sternum as a function of screw-plate fixation techniques
- Evaluated the cyclic fatigue resistance of porcine sterna secured with cortical bone screws and plates versus cancellous bone screws and plates

2005

Junior Interactive Qualifying Project (IQP),
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Aquaculture of the Kabeljou in Namibia

Mentor: Professors Bland Addison and Stephen Weiniger

- Made recommendations for lowering the costs of a land-based kabeljou aquaculture industry to the Ministry of Fisheries and Marine Resources of Namibia
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2005

Research Experiences for Undergraduates (REU) Fellow,
Department of Mechanical Engineering, University of Minnesota,
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Functional Corneal Engineering: Layered Collagen Film-Based Stroma Equivalents

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HONORS, AWARDS AND FELLOWSHIPS

2009	Sigma Xi Research Society Induction
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PUBLICATIONS

Refereed journal Articles:

1. **D. Lavin***, M. Harrison*, L. Tee, K. Wei, and E. Mathiowitz. A novel wet extrusion technique to fabricate self-assembled microfiber scaffolds for controlled drug delivery. *Journal of Biomedical Materials Research Part A*, in press, 2012.
2. **D. Lavin**, R. Stefani, L. Zhang, S. Furtado, R. Hopkins, and E. Mathiowitz. Multifunctional polymeric microfibers with prolonged drug delivery and structural support capabilities. *Acta Biomaterialia*, (doi:10.1016/j.actbio.2012.01.019), 2012.
3. R. Patel*, D. Cho*, C. Tian, A. Chang, K. Estrellas, **D. Lavin**, S. Furtado, and E. Mathiowitz. Doxycycline delivery from PLGA microspheres prepared by a modified solvent removal method. *Journal of Microencapsulation*, (doi:10.3109/02652048.2011.651499), 2012.
4. R. Crabb, E. Chau, **D. Decoteau**, and A. Hubel. Microstructural Characteristics of Extracellular Matrix Produced by Stromal Fibroblasts. *Annals of Biomedical Engineering*, 34(10): 1615-27, 2006.

Non-refereed journal articles:

1. R. Patel, D. Cho, C. Tian, A. Chang, **D. Decoteau**, J. Morgan, E. Mathiowitz. A novel W/O/O double-emulsion solvent removal method for the nanoencapsulation of doxycycline. *CRS Society Newsletter*, 28(2): 18-19, 2011.
2. **D. Decoteau**, D. Flannery, A. Hart, H. Zec, R. Dunn, K. Billiar. Cyclic failure analysis of sternal fixation techniques. *Proceedings of the IEEE 32nd Annual Northeast Bioengineering Conference*, 01(02): 105-106, 2006.

Manuscripts in progress:

1. **D. Lavin**, L. Zhang, S. Furtado, J. Reineke, R. Hopkins, and E. Mathiowitz. Effects of protein molecular weight on the release kinetics and intrinsic material properties of wet spun polymeric microfibers. Submitted to *Acta Biomaterialia* (*in review*).

(Manuscripts in progress – continued)

2. **D. Lavin**, L. Zhang, S. Furtado, R. Hopkins, and E. Mathiowitz. Designing wet spun polymeric monofilaments and multifilament yarns as controlled-release drug delivery systems for biomedical applications. Submitted the Journal of Controlled Release (*in review*).
3. **D. Lavin**, L. Zhang, R. Quinn, S. Hilbert, A. Bert, J. Bustamante, K. Merrigan, S. Neighbors, C. Thanos, E. Mathiowitz and R. Hopkins. Evaluation of tissue-engineered sutures for the prevention of fibrocalcifications in cardiovascular reconstructive surgeries (*anticipated*).
4. **D. Lavin**, S. Bakhru, S. Furtado, L. Patel, R. Hopkins, and E. Mathiowitz. Designing spatially distinct polymeric microfibers with tunable drug delivery (*anticipated*).

Abstracts and conference proceedings:

1. **D. Decoteau**, D. Cho, L. Zhang, R. Stefani, R. Patel, and E. Mathiowitz. Single step fabrication of wet spun binary phase composite microfibers. 38th Annual Meeting and Exposition of the Controlled Release Society, National Harbor, MD, July 2011.
2. **D. Decoteau**, L. Zhang, R. Stefani, R. Hopkins, and E. Mathiowitz. Effect of protein molecular weight on release from wet spun PLGA and PLLA fibers fabricated by phase-inversion. Biomedical Engineering Society Annual Meeting, Austin, TX, October 2010.
3. **D. Decoteau**, R. Stefani, L. Zhang, M. Harrison, R. Hopkins, and E. Mathiowitz. Encapsulation, in vitro drug release, pH changes and tensile strength characteristics of wet spun poly(L-lactic acid) microfibers. 37th Annual Meeting and Exposition of the Controlled Release Society, Portland, OR, July 2010.
4. R. Patel, D. Cho, C. Tian, A. Chang, **D. Decoteau**, J. Morgan, and E. Mathiowitz. A novel W/O/O double emulsion solvent removal method for the nanoencapsulation of doxycycline. Annual Meeting and Exposition of the Controlled Release Society, Portland, OR, July 2010.
5. **D. Decoteau**, A. Navarro, L. Zhang, R. Stefani, R. Perez, E. Mathiowitz, and R. Hopkins. Drug delivery sutures for the prevention of fibrocalcifications in tissue-engineered cardiovascular constructs. 4th Biennial Heart Valve Biology and Tissue Engineering Meeting, Hilton Head, SC, March 2010.
6. B. Bilgen, **D. Decoteau**, Y. Ren, C. MacKay, K. Aslani, B. Fleming, E. Mathiowitz, R. Aaron, D. and M. Ciombor. TGF- β 1 delivery by PLGA microspheres in hypoxia for chondrogenesis. 56th Annual Meeting of the Orthopaedic Research Society, New Orleans, LA, March 2010.

(Abstracts and conference proceedings – continued)

7. **D. Decoteau**, M. Harrison, R. Hopkins, and E. Mathiowitz. Sequential release of dexamethasone from poly(L-lactide) microfibers. 36th Controlled Release Society Annual meeting, Copenhagen, Denmark, July 2009.
8. **D. Decoteau**, M. Harrison, R. Hopkins, and E. Mathiowitz. Biodegradable sutures for localized drug delivery. Poster Presentation, 8th World Biomaterials Conference, Amsterdam, The Netherlands, May 2008.
9. M. Harrison, **D. Decoteau**, Y. Tee, and E. Mathiowitz. Fabrication of drug-loaded microfibers via solvent removal. Poster Presentation, 34th Annual Meeting and Exposition of the Controlled Release Society, Long Beach, CA, July 2007.
10. **D. Decoteau**, D. Flannery, A. Hart, H. Zec, R. Dunn, and K. Billiar. Cyclic failure analysis of sternal fixation techniques. 32nd Northeast Bioengineering Conference, Easton, PA, April 2006.

U.S. PATENTS

Methods, Compositions and Kits for Therapeutic Treatment with Wet Spun Binary Phase Microstructures. U.S. Provisional Application No. 61/513,470. Filed 7/29/11.

TEACHING EXPERIENCE

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| 2010 | Teaching Assistant , Brown University, Providence, RI
<i>BIOL 2240 Artificial Organs, Biomaterials, and Tissue Engineering Seminar</i> (Various Instructors) <ul style="list-style-type: none">• Organized graduate student presentation schedule• Facilitated anonymous feedback between students and faculty |
| 2008 | Teaching Assistant , Brown University, Providence, RI
<i>BIOL 2130 Molecular Techniques in Cell Biology</i> (Professor Jeffrey Morgan) <ul style="list-style-type: none">• Taught graduate and undergraduate level students various techniques in molecular and cell sciences• Coordinated and proctored laboratories twice a week• Managed course website• Revised and updated laboratory protocols |

(Teaching Experience – continued)

- 2007, 2009 **Teaching Assistant**, Brown University, Providence, RI
BIOL 2110 Drug and Gene Delivery (Professor Edith Mathiowitz)
- Taught graduate and undergraduate level students concepts in drug delivery
 - Organized guest lectures and maintained course website
 - Graded homework, student presentations and final papers

PEDAGOGICAL TRAINING

- 2011 – 2012 **Teaching Certificate III Recipient**, The Harriet W. Sheridan Center for Teaching and Learning, Brown University, Providence, RI
- Professional development seminar focused around the development of a teaching portfolio designed to help participants prepare for the academic job market and teaches participants to document the scholarship of their teaching
- 2011 – 2012 **Teaching Certificate II Recipient**, The Harriet W. Sheridan Center for Teaching and Learning, Brown University, Providence, RI
- Classroom tools seminar that builds on Certificate I focused on the design, implementation, and assessment of a reflective teaching
- 2009 – 2010 **Teaching Certificate I Recipient**, The Harriet W. Sheridan Center for Teaching and Learning, Brown University, Providence, RI
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INVITED LECTURES

- 2011 Cardiac Surgery Lecture Series, Children’s Mercy Hospital, Kansas City, MO. *Polymer Implants for Circulatory Devices*
- 2009, 2010 Drug and Gene Delivery Guest Lecture, Brown University, Providence, RI. *Fiber-based Drug Delivery Systems*

(Invited Lectures – continued)

- | | |
|------|--|
| 2009 | Veterans Affairs Repair and Regeneration Conference,
Providence, RI.
<i>Sequential Release of Dexamethasone from Poly(L-lactide)
Microfibers</i> |
| 2008 | Cardiac Surgery Lecture Series, Children’s Mercy Hospital,
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MENTORING EXPERIENCE

Master’s Thesis:

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| 2011 | Linda Zhang, Biology |
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Honors Theses:

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| 2010 | Amy O’Brien, Biology |
| 2010 | Ashley Pender, Biology |
| 2010 | Robert Stefani, Biomedical Engineering |
| 2010 | Linda Zhang, Biology |

Fellowship Awards: *[Karen T. Romer Undergraduate Teaching and Research Award (UTRA) and National Science Foundation Research Experience for Undergraduates (NSF REU) programs]*

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| 2009 | Linda Zhang, Biology, 2009 (UTRA fellowship) |

SERVICE

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| 2010 – 2011 | BioMed Representative, Brown Graduate Biomedical Engineering Society |
| 2010 | Volunteer, Brown University NSF GK12 Program, Vartan Gregorian Elementary School Lecturer |
| 2004 – 2006 | Industrial Liaison, WPI Biomedical Engineering Society |
| 2005 – 2006 | Volunteer, St. Vincent Hospital Emergency Department, Worcester, MA |

PROFESSIONAL SOCIETY MEMBERSHIPS

2010 – American Association for the Advancement of Science (AAAS)
2010 – Biomedical Engineering Society (BMES)
2007 – Controlled Release Society (CRS)
2004 – Society of Women in Engineering (SWE)

RESEARCH INTERESTS

Drug Delivery
Nanomedicine
Micro/Nanoencapsulation
Translational Research
Tissue Engineering
Regenerative Medicine

Preface

The primary motivation for this work was to develop multifunctional polymeric fibers that could be used as localizable drug delivery interventions to improve the surgical outcomes of implantable biomaterials and tissue engineered constructs. These applications have tremendous importance in the fields of drug delivery, polymer science, and materials processing.

The chapters in this thesis occur sequentially by their relevance to the previous chapter. Chapters 2 through 6 are written in manuscript style and include the following sections: introduction, materials and methods, results, discussion, conclusions, and references. Chapter 1 will serve as a background and will discuss the common methods used to fabricate polymeric fibers, and outline the advantages and disadvantages of each method in the design of multifunctional delivery systems. In addition, I will justify the selection of the wet spinning technique for this thesis and provide an extensive review of the current applications and physical properties of wet spun fibers.

The five remaining chapters address the specific aims of this research. The primary aim of Chapter 2 was to develop a new wet extrusion technique that would allow for the formation of wet spun filaments within the diameter ranges desired for soft tissue engineering applications. In addition, I sought to show how useful this new technique is for controlled drug delivery by evaluating two different drug encapsulation techniques capable of providing immediate and prolonged delivery of heparin. This study is in

revision and has been resubmitted to the Journal of Biomedical Materials Research Part A.

Chapter 3 describes the fabrication of stable, monofilament fibers capable of providing both controlled release and mechanical support. In this chapter, a model hydrophobic drug, dexamethasone, was encapsulated into wet spun fibers by phase inversion and the dynamic mechanical properties of single fibers are evaluated. In addition, some correlations are made between wet spinning conditions (solvents, nonsolvent, polymer precipitation strength, etc.) and the mechanical strength of resulting filaments. This study has been published in *Acta Biomaterialia*.

Chapter 4 discusses the effects of protein molecular weight on the intrinsic material properties of poly(L-lactic acid) (PLLA) and poly(lactic-co-glycolic) (PLGA) fibers. Insulin, lysozyme, and bovine serum albumin (BSA) were encapsulated within PLLA and PLGA fibers and the tensile properties and release profiles were quantified. As well, the mechanism of protein release was investigated using the Korsmeyer-Peppas kinetic model. This study has been submitted to *Acta Biomaterialia*.

Chapter 5 presents the scale up of individual monofilaments into multifilament yarns. This chapter focused on understanding what wet spinning conditions are optimal for the development of drug delivery sutures, taking into consideration the lessons learned in Chapters 3 and 4. As well, the biological activity of encapsulated dexamethasone was evaluated up to 56-day incubation. Chapter 5 emphasizes the complexity of developing multifunctional wet spun fiber delivery systems. This study has been submitted to the *Journal of Controlled Release*.

Chapter 6 investigates the application of drug eluting yarns as drug delivery sutures in an *in vivo* ovine model for vascular calcification. Drug-eluting yarns prepared using the methods described in Chapter 5 were hybridized with existing surgical sutures positioned between the anastomoses of valve conduits implanted in the right ventricular outflow tract of juvenile sheep. Microscopy studies were performed to evaluate the

biocompatibility of drug-eluting yarns at 20 weeks post-implantation. Radiography, inductively coupled plasma optical emission spectrometry, and quantitative reverse transcriptase polymerase chain reaction studies were also used to evaluate cellular responses to suture anastomoses with and without drug treatment.

Chapter 7 summarizes the results presented in Chapters 2 through 6, and presents the future directions and recommendations for this work.

Appendix A focused on the development of spatially distinct polymeric fibers. The characterization of these fibers—including cross-polarized light microscopy, differential scanning calorimetry, scanning electron microscopy, and release kinetics of a model protein, BSA—is also presented in Appendix A.

Acknowledgements

I would first like to thank my advisor, Edith Mathiowitz for allowing me to join her lab. Over the course of the past six years, I have gotten to know how truly brilliant she is as a scientist, and have a tremendous amount of respect for her and the knowledge she has bestowed upon me. Thank you Edith for teaching me how to be an independent scientist and balance life outside the lab.

I would also like to thank Dr. Hopkins for coming to Edith with the idea of creating a drug delivery suture. I could not have asked for a better project, or a more enthusiastic mentor. Dr. Hopkins, your passion for science is infectious, and I am very grateful to have had the opportunity to work with you. Thank you for all your guidance and support throughout this project.

Thank you also to the rest of my thesis committee—Diane Hoffman-Kim, Jeffrey Morgan, and Anubhav Tripathi—for their willingness to read my work and for providing valuable insights throughout my time at Brown.

I would especially like to thank the other members of my lab who offered much guidance and support during my early years in graduate school. I am indebted to Mike Harrison for showing me around the lab and giving me my first introduction to polymer science and drug delivery. As well, I would like to thank Peter Morello, Ana Jaklenec, Peter Cheifetz, and Josh Reineke, for their valuable feedback and suggestions in the beginning stages of this project. Thank you also to Chris Thanos for all his help and advice with the histology studies and Stacia Furtado for all her help with x-ray studies.

I also appreciate all the other members of the lab (past and present) for their technical assistance over the years.

I want to take the opportunity to thank Linda Zhang for all her help, which was especially critical for the animal implant studies. Linda, thank you for being so awesome and for fueling me with caffeine and sweets throughout the late nights we spent working in the lab together into the wee hours of the morning. As well, I'd like to thank the rest of the undergrads that helped me throughout the various stages of this project: Robert Stefani, Ashley Pender, and Amy O'Brien.

I'd also like to thank all the people at Children's Mercy Hospital who helped with this work. A huge thank you to the surgery team—Dr. Bert, Dr. Hilbert, Rachael Quinn, Stacy Neighbors, Julie Bustamante, Kellie Merrigan—and others who helped me during my summer in Kansas City—Maria Navarro, Rick Perez, and Chris McFall.

I would also like to thank the administrative staff at Brown—Carol Folan and Cheryl Pariseau and Children's Mercy Hospital—Mary Bailey and Annalisa Deaton for helping me throughout the way. As well, the rest of the third floor crew for their company and technical assistance throughout the years.

I want to thank my friends, Courtney Nowill, Sarah Stout, Helena Zec, Marcella Corcoran, and Whitney Moore-Steele for their unwavering support throughout graduate school. Thank you for being so understanding when I could not make it to social events and for routing me on towards the end...I am truly thankful to have friends like you!

I owe a great deal of thanks to my family—words cannot express how blessed I am to have such a support system in my life. Thank you Mom, Dad, Stephen and Kirsten for encouraging me to go on, and lifting me up when I needed it most. Also thank you to Amélie, Laruienne, Étienne, Andy, and the newest member of my family, my nephew Andrew, for all the love and support you've given me. I also would like to thank my in-

laws who have become my other family—Judy, John, Lindsie, Sean, Gram, and Pa—for all their love and encouragement.

I am also thankful for my cat, Figaro, who kept me company while writing this thesis.

Last, but certainly not least, I would like to thank my husband and high school sweetheart, Bryan for his continuous support through both the good times and the bad times of graduate school. Bryan, thank you for being the absolute best ‘house husband’—especially for doing all the laundry, cleaning, and cooking while I sat and wrote this thesis. Without you, none of this would have ever been possible. I love you.

Dedication

I dedicate this thesis to my father—for teaching me the importance of education at a young age—and to my mother, who showed me what it means to have a relentless work ethic.

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Chapter 1

Introduction

1.1 Background and significance

Fibers have long been used in the medical field to aid in maintaining and improving the quality of life. Applications of fibers range in degree of complexity from simple surgical sutures, to prosthetic implants and medical devices. Fiber based materials or textile structures are advantageous to biomedical applications because the surface-to-volume ratio is very large, which is important for surface-mediated applications such as kidney dialysis devices. Fibers can also be engineered into woven, knitted, braided or hollow structures to provide varying degrees of porosity and strength needed for biomedical applications such as synthetic blood vessels, hernia repair meshes, and dental grafts. It is therefore not surprising that fibers play a major role in emerging tissue engineering and regenerative medicine strategies as scaffolds and substrates for the reconstruction of tissues [1].

Although many biomedical implants perform adequately for years, the long-term success of implanted biomaterials is severely limited by a dynamic host inflammatory response, which often leads to infection, rejection and recurrence [2, 3]. Designing tissue engineered constructs to improve the integration of scaffold substrates and the

regeneration of damaged tissues/organs while satisfying physiological functions remains an unmet challenge. Moreover, the lessons learned from one cell/tissue type are often site-specific and cannot readily be applied to another therapeutic target. Nevertheless, with 25 million people in the US relying on implanted medical devices with an estimated 2 million new devices implanted in patients each year [4], the clinical demand for the development of next generation devices based on new technologies and improved materials is needed.

Building drug delivery into existing biomedical textiles and fiber based scaffolds is one approach to improve the functionality of implants by supplying a locally directed therapy to reduce biomaterial-driven inflammation and enhance tissue regeneration. Polymer based controlled release technologies can also be optimized for the spatial and temporal release of multiple essential factors specific to therapeutic targets. Multifunctional polymeric fibers could be extremely useful in the design of novel biomedical implants and tissue engineered scaffolds by providing immediate mechanical support at the implant site and concurrently delivering therapeutics to enhance biological responses.

1.2 Methods of fabricating polymeric fibers

There are several methods for fabricating natural or synthetic polymer materials into monofilament fibers, the most common of which are melt spinning, dry spinning, wet spinning and electrospinning. All of these techniques involve the extrusion of a polymer melt or solution. The advantages and limitations associated with each technique are described in the following subsections, and the selection of wet spinning for this thesis is supported.

1.2.1 Melt spinning

In melt spinning, a polymer is heated to its melting temperature to form a molten polymer solution that is then forced through a die drilled with single or multiple holes, termed a spinneret. Each die in the spinneret creates a single filament. The liquid polymer melt is then typically drawn through a series of cooling gases and/or cooling baths by a take-up wheel to impart strength on solidifying filaments by stretching to orient polymer chains along the fiber axis (**Figure 1.1**). Melt spun fibers have high mechanical strength in comparison to other spinning methods and can be extruded with different cross-sectional morphologies (round, trilobal, pentagonal etc.) by altering die geometry. Fibers produced from polymer melts are limited to materials with melting temperatures and can sometimes undergo thermal degradation due to processing at high temperatures [5, 6]. Synthetic thermoplastic polymers, such as polypropylene and polyester, are commonly produced in this manner.

Although filaments are drawn down during solidification and finishing processes, fiber diameters are usually not smaller than 10 μm . A variation of this fabrication technique, termed melt blowing, has been used in industrial applications to further reduce the diameter of filaments extruded from polymer melts to 1-2 μm [7]. Melt blown fibers are produced in a single step by extruding a polymer melt through a v-slotted die using a jet of hot air. The resultant drag force on the molten polymer solution transforms the melt into fibers that are collected on a mat located away from the die.

Surgical sutures and woven or knitted medical textiles are the most common application of melt spun fibers. Melt spun fibers have also been evaluated as three-dimensional scaffolds and superstructures for various tissue engineering applications [8-10]. Melt spinning is not suitable for the encapsulation of heat-sensitive proteins and other biotherapeutics due to the potential for loss of biological activity during processing. For example, the extrusion temperatures of melt spun poly(L-lactic acid) (PLLA) filaments can be as high as 240°C [11], and most proteins denature around 50–60°C. The

incorporation of drugs into melt spun fibers has been achieved by encapsulating drugs with high melting temperatures [12], coating existing melt spun fibers/meshes with polymer slurries containing drugs [13-16], or hybridization of melt spun fibers and polymeric microspheres [17]. The release profiles of drugs delivered from melt spun fibers extend from days to weeks.

1.2.3 Dry spinning

Dry spinning begins with a polymer dissolved in a solvent to form a solution called the spin dope (**Figure 1.2**). The spin dope is extruded through a spinneret similar to melt spinning. As the spin dope solution exits the spinneret, heated air and/or gas is introduced into the spinneret zone to remove the solvent from the polymer solution stream to form a solid filament [5]. ‘Direct write’ [18, 19], and ‘spinneret based tunable engineered parameters (STEP)’ [20, 21] are similar to dry spinning and involve the formation of polymeric fibers using air to evaporate solvent from a spin dope solution. ‘Dry-jet-wet’ spinning is yet another common variation of traditional dry spinning. Fibers made using the dry-jet-wet spinning technique are first dry spun and then passed through a series of coagulation baths to further solidify filaments [22, 23]. The diameters of fibers produced using these methods range from several hundred micrometers to as small as 50 nm.

Dry spun fibers have high mechanical strength similar to melt spun fibers and are also used in the design of knitted mesh scaffolds for medical applications [23]. Ultra-fine fibers produced by direct write and STEP are used for tissue engineering applications only, and have been studied as morphological guides for directed cellular growth and differentiation [19-21]. Dry spinning is the least studied technique for drug delivery applications. Although dry spinning may be done at ambient temperatures, the evaporation of solvent typically occurs under high temperatures to facilitate extraction, making them less suitable for protein encapsulation. Dry spinning is also limited to

polymers that dissolve in volatile solvents and cannot be used for spinning natural polymers. In the textile industry, this technique is only used for polymers which cannot be melt spun due to safety and environmental factors associated with the handling of volatile solvents [5]. Acetate and spandex are the most common dry spun textile fibers.

1.2.4 Wet spinning

Wet spinning is the most complicated method of fiber spinning and begins with dissolving a polymer in a suitable solvent. Spin dope solutions used for wet spinning have lower polymer concentrations in comparison to melt spinning and dry spinning techniques since the solution is spun at lower temperatures. Once the polymer has been fully dissolved, it is extruded thru a spinneret and into a nonsolvent coagulation bath (**Figure 1.3**). Since the solvent is miscible with the nonsolvent bath and the polymer solution is not, a continuous liquid polymer stream precipitates into a solid filament. The amount of time the fiber is left in the coagulation bath is referred to as the residence time. Fiber structure and shape are determined during the residence time and gelation of the polymer spin dope due to complex interactions of many factors which affect the phase separation of the polymer solution (solvent/nonsolvent miscibility parameters, precipitation strength, residence time, extrusion rate etc.) [5, 6]. Rayon and spandex are common materials used in wet spinning.

From a drug delivery perspective, wet spinning is most similar to microsphere based encapsulation techniques and is advantageous for the encapsulation of water-soluble drugs since both the solvent and coagulant can be non-aqueous. Wet spinning can also be used to spin natural polymer materials such as chitosan and alginate. The diameter of filaments produced by wet spinning is around 30–200 μm [6, 24-27]. Another variation of this technique, referred to as ‘hydro-spinning’, can be used to create irregular ribbon-like fibers with diameters as small as 4–10 μm by the extrusion of spin dope solutions into a stirring nonsolvent coagulation bath [28, 29]. The applications of

wet spun fibers in drug delivery and tissue engineering is discussed in more detail in section 1.3.

1.2.5 Electrospinning

Electrospinning is used to create ultra-thin fibers ranging from 3 nm to 10 μm in diameter. The electrospinning technique relies on electrostatic interactions to draw a polymer solution into fibers. In this method, a voltage is applied to the spin solution until a repulsive charge is large enough to overcome the attractive force of surface tension holding the polymer to the spinneret tip. The polymer solution is then transformed into a polymer jet and drawn into thin fibers towards a grounded collector (**Figure 1.4**). The solvent from polymer solutions evaporates from the polymer jet before reaching the collecting plate [30, 31].

Electrospinning can be used to produce natural and synthetic polymeric fibers and is frequently used in many tissue engineering applications since the small diameters of electrospun fibers closely mimic the size scale of extracellular matrix (ECM) fibers. Common electrospun materials include poly(ϵ -caprolactone) (PCL) [32, 33], polyurethane (PU) [34], poly(L-lactic acid) (PLLA) [35, 36], and poly(lactic-co-glycolic acid) (PLGA) [37, 38]. Biomimetic scaffolds made from electrospun fibers have been studied extensively for bone, cartilage, and tendon and ligament, neural, and cardiovascular tissue engineering [30].

Electrospun fibers are transformed from a polymer melt or a polymer solution referred to as the dissolving method. The dissolving method is the traditional method of electrospinning since it can be used to encapsulated thermo-sensitive drugs, whereas the melt electrospinning technique is more suitable for industrial production of nonwoven meshes and felts. Electrospun fibers have also been evaluated as drug delivery vehicles to reduce inflammatory responses to implanted neural devices [37] and to prevent post-

surgical adhesions and infection [39]. Although electrospinning lends itself to drug delivery technologies, asserting the control over the initial burst of water-soluble therapeutics continues to be a difficult task due to high surface-to-volume ratios [40, 41].

1.3 Applications of wet spun fibers

Despite the ability to encapsulate heat-sensitive therapeutics using electrospinning techniques, electrospun fibers offer little structural support as single filaments. In recent studies, co-axial spinning has been investigated for the scale-up of electrospun fibers into micro-scale threads to enhance mechanical strength [42, 43]. This thesis focuses on the development of drug-eluting fibers capable of physical manipulation for surgical reconstructions and the design of macro-level scaffolds. Thus, wet spinning was selected to fabricate polymeric monofilaments because it is most similar to conventional microsphere based drug encapsulation techniques, avoids the potential for thermal denaturation of proteins unlike melt spinning and dry spinning methods, and produces filaments within the size ranges of surgical sutures. The remainder of this section is dedicated to providing the reader with information on the applications currently associated with wet spun fibers.

1.3.1 Tracks for guiding cell behavior

The ability to precisely control the attachment, migration, alignment, proliferation, and differentiation of cells is extremely important for the regeneration of tissues. Wet spun fibers have been investigated by several groups as tracks to guide and direct the behavior of cells for a variety of applications, such as neuronal tissue engineering, musculoskeletal tissue engineering, vascular tissue engineering, and wound healing.

Controlling the migration and alignment of cells has important implications in tissue regeneration after spinal cord or peripheral nerve injury. For these reasons, most *in vivo* studies involving the use of fibers to direct cellular migration and differentiation fall within neuronal tissue engineering applications. PLLA fibers (60–80 μm) placed inside nerve guidance channels have been shown to enhance axon growth and maturation in an *in vivo* rat sciatic nerve model [44]. Kang et al. also showed neurite extensions from primary rat neurons had greater alignment on micron-grooved alginate fibers (100–200 μm) when compared to smooth fibers *in vitro* [45].

PLA:PLGA fibers (25–35 μm) aligned on conducting platforms [46], and PLLA fibers (60 μm) coated with extracellular matrix proteins [47] have demonstrated great potential for guiding the orientation and differentiation of muscle cells *in vitro*. As well, fibrin (50–100 μm) [48], and PLGA (10–250 μm) [49] fibers evaluated as guides for wound healing enhanced attachment and alignment of fibroblasts. The physical properties of filaments have also been found to influence cell behavior; the enhanced proliferation of human umbilical vein endothelial cells cultured on wet spun PCL fibers (150 μm) was greater on post-drawn fibers compared to as-spun fibers for vascular tissue engineering applications [50].

1.3.2 Three-dimensional (3D) scaffolds for tissue engineering

Different polymers, such as collagen, PLLA, PCL, and polyurethane (PU) have been proposed as wet spun fibrous scaffolds for different tissue engineering applications. For example, wet spun polyphosphazene and PLLA fiber blends (50–80 μm) used in bone tissue engineering have demonstrated enhanced osteoblast adhesion and growth compared to tissue culture plastic substrates [51]. Wet spun fibers have also been evaluated for use as vascular grafts and soft tissue repair. Caves et al. used aligned collagen fibers (40 μm) imbedded within multiple layers of an elastin-mimetic protein

polymer to tune the mechanical properties of tubular vascular grafts [52] and abdominal wall grafts [53].

Recent studies have used wet spun fibers in combination with electrospun fibers to improve the mechanical support of scaffolds used in bone tissue engineering. Tuzlakoglu et al. electrospun type I collagen fibers onto starch-based wet spun fibers (40 μm), which improved the metabolic activity of osteoblasts and resulted in cellular orientations resembling the microcapillary-like organizations seen during angiogenesis [54]. Williamson et al. also fabricated hybrid PCL-PU composites by electrospinning PU fiber backing onto wet spun PCL fibers (~ 100 μm) to support the formation of stable functional endothelial cell monolayers for vascular tissue engineering applications [55].

1.3.3 Vehicles for localized drug delivery

A broad range of biotherapeutics including antibiotics [14, 56, 57], heparin [58], proteins [45, 47, 59-61], growth factors [62, 63], genes [64, 65], viruses [66, 67], and even stem cells [68], have been successfully incorporated into wet spun fibers for many biomedical applications. Polymer fiber delivery systems have been achieved by impregnating therapeutics into the core of hollow fibers, entrapping therapeutics within fibers, and chemically crosslinking or adsorbing therapeutics to the surfaces of fibers. The high surface area to volume ratio of wet spun fibers is advantageous for mass transfer and efficient drug release.

The effective delivery of therapeutics from various wet spun delivery systems has been demonstrated. Release of a model protein, bovine serum albumin (BSA), from the hydrogel cores of co-extruded wet spun PLLA fibers fabricated by Crow et al. exhibited sustained delivery up to eighty days *in vitro* [60]. Gao et al. also achieved the sustained delivery of a model hydrophilic anti-cancer drug, 5-fluorouracil, impregnated within wet spun PLLA fibers up to twenty-one days *in vitro* [69]. A recent study by Jung et al.

even showed that the delivery of cell-permeable gene complexes from PLLA scaffolds improved the transfection of stem cells attached to the surfaces of fibers in comparison to bolus delivery strategies [64]. Wet spun fibers have also been approved as ocular drug delivery systems for treatment of posterior-segment diseases, such as macular edema [70].

1.4 Properties of wet spun fibers

Wet spun filaments are drawn similar to melt and dry spun fibers in the textile industry to obtain desired mechanical strength, elasticity, and elongation. The parameters affecting the properties of filaments during mechanical stretching are well known [22, 23, 71-73]. During filament drawing, polymer chains are oriented along the fiber axis resulting in a reduction in fiber diameter and increase in tensile strength. Over the past decade, the use of wet spun fibers in tissue engineering and drug delivery technologies has substantially increased. The mechanical properties of filaments used in these applications are presented and the selection of PLLA and PLGA for this work is justified.

1.4.1 Drug-free fibers

Kulkarni et al. were among the first to wet spin PLA fibers as early as 1966 [74]. The use of wet spun fibers in the textile industry is not generally used since wet spun filaments are not as strong or cost-effective as those produced by melt spinning methods. However, the use of wet spun fibers in the design of tissue engineered scaffolds for example, does not require the same mechanical strength as melt spun filaments used in the design of sails and parachutes.

Nelson et al. presented a simple and repeatable bench top technique to fabricate monofilament fibers for use as tissue engineering scaffolds [24]. They produced fibers (30–550 μm) wet spun from PLLA and PLGA and coagulated in a number of nonsolvents, including isopropyl alcohol, heptane, pentane, and hexane. The reported tensile strengths from their work varied with fiber draw ratio and ranged from 30 MPa to 120 MPa. Draw ratio is a measure of the degree of stretching during filament drawing and was defined as the ratio of the velocity of the fiber on the take-up wheel to the velocity of spin dope extrusion.

Williamson et al. have also studied the material properties of PCL wet spun filaments (150–200 μm) for tissue engineering applications [26]. They used a simplified spinning apparatus, which consisted of a syringe and single spinneret tip positions above a methanol coagulation bath. The PCL spin dope solutions in their modified wet spinning method were allowed to flow under gravity through the spinneret into the nonsolvent bath and were either cold-drawn after precipitation or left as-spun. The reported tensile strength from their work also increased with fiber drawing similar to Nelson and colleagues [24], ranging from 1 MPa to 48 MPa.

The mechanical properties of fibers wet spun from collagen and regenerated silk have also been evaluated. As-spun collagen fibers ($\sim$ 30–60 μm) manufactured by Cornwell et al. had tensile strengths from 2 MPa to 45 MPa [75], whereas post-drawn regenerated silk fibers ($\sim$ 10–20 μm) produced by Yan et. al had tensile strengths from 120 MPa to 420 MPa [27].

1.4.2 Drug-loaded fibers

The combination of controlled release technologies and tissue engineering strategies are synergistic for the regeneration of tissues that require a complex sequence of biological cues and structural support. However, the majority of research involving wet spun fibers

has primarily focused on the release of therapeutics or biocompatibility and tissue regeneration capabilities of 3D scaffolds. Little is known about the effects of drug encapsulation on the material properties of wet spun fibers.

Research groups that have evaluated the mechanical properties of drug-eluting wet spun microfilaments focused on the effects of encapsulating small molecules (< 1 kDa) or model proteins of similar molecular weight. Mack et al. encapsulated dexamethasone (392.5 Da) into wet spun PLGA fibers (250–300 μm) with the highest tensile strengths around 7-10 MPa [76]. Chang et al. encapsulated a small molecular weight molecule, gentamicin sulphate (463.6 Da) into PCL fibers (170–220 μm) [77]. The processing conditions used in their study produced drug-eluting PCL fibers with tensile strengths from 4 MPa to 8 MPa. The tensile strengths of PCL fibers (150–200 μm) loaded with ovalbumin (45 kDa) and have also been evaluated. PCL fibers loaded with albumin had tensile strength of around 7 MPa and resulted in a 160% reduction in elongation in comparison to PCL fibers without drug loading. Rissanen et al. studied the effect of drug loading on poly(L,D-lactic acid) fibers (50–70 μm) using a protein of similar molecular weight, bovine serum albumin (BSA, 66 kDa) [25]. Fibers encapsulating BSA had strengths of around 7 and 17 MPa, dependent on processing conditions.

1.5 Specific Aims

There is a compelling need for the development of ‘next generation’ devices based on new technologies and improved materials. The motivation behind my work is to design polymeric fibers that can be used as drug delivery vehicles for biomedical applications, such as sutures, scaffolds, and localizable delivery systems. Accordingly, the specific aims of my thesis are four-fold:

Specific Aim 1. To develop novel wet spinning methods to fabricate drug-eluting polymeric fibers.

A number of wet spinning methods and variants have been used to create polymeric fibers. However, previous studies have mostly looked at the use of wet spun filaments as drug delivery vehicles or guides for tissue regeneration. New techniques are needed for the translation of wet spun filaments into multifunctional drug delivery systems that can both perform a mechanical function and simultaneously deliver drugs. The size of polymeric fibers has been shown to affect a number of cellular responses, including binding, spreading, orientation, and motility [78]. Alternative approaches to fabricate wet spun polymeric scaffolds with smaller average diameters would also broaden the scope and application of wet spun filaments in soft tissue engineering applications where small-scale features are important.

Wet spinning is a nonsolvent-induced phase inversion technique that results in the phase separation of polymeric solutions through immersion precipitation. Phase separation fundamentals are the basis of many fabrication techniques in controlled release technologies and tissue engineered scaffolds. The objective of this research is to develop new and improved methods for fabricating multifunctional wet spun fibers by altering the key parameters governing phase separation (polymer type, polymer concentration, solvent/nonsolvent miscibility parameters, etc.).

Specific Aim 2. To encapsulate and deliver therapeutic molecules (hydrophobic and hydrophilic) from wet spun fibers at biologically relevant rates.

The success of designing multifunctional wet spun fibers lies in achieving both structural support and controlled drug delivery. Fibers used for ‘next generation’ tissue engineering strategies should have the ability to deliver therapeutics with a wide range of molecular

weights and chemical properties. Model hydrophobic (dexamethasone) and hydrophilic (heparin, insulin, lysozyme, noggin, bovine serum albumin) molecules will be encapsulated within wet spun filaments using the phase inversion methods developed in Aim 1. Another main objective of this aim is to study the effect of processing conditions on release kinetics and biological activity. Poly(L-lactic acid) and poly(lactic-co-glycolic acid) will be evaluated as fiber drug delivery vehicles since they have FDA approval and are two of the most commonly used polymers in biomedical engineering and drug delivery. Other hydrophilic polymers and excipients will also be studied to modulate the release of encapsulated therapeutics. Lastly, the mechanism of drug release from wet spun filaments will be evaluated by applying kinetic models to experimental release profiles.

Specific Aim 3. To evaluate the intrinsic material properties of wet spun fiber delivery systems as they apply to biomedical and tissue engineering applications.

As previously discussed, little is known about the effects of drug encapsulation on the tensile strength of wet spun fibers. For wet spun fibers to be useful in the fabrication of tissue engineering scaffolds and biomedical implants, the effects of drug encapsulation on the materials properties must be studied. The goal of this research is to understand the effects of processing conditions on the mechanical properties of wet spun delivery systems fabricated in Aim 2. Uniaxial tensile testing will be performed to characterize the mechanical integrity of wet spun filaments and to further substantiate the relationship between fabrication parameters and drug encapsulation. The thermal characteristics and structure of wet spun filaments will also be determined using x-ray diffraction and differential scanning calorimetry characterization methods to aid in this understanding.

Specific Aim 4. To assess the therapeutic potential of bioengineered fibers for use as drug delivery sutures *in vivo*.

Although sutures are among the earliest recorded medical devices and have remained the primary method of wound closure for thousands of years [79], the process of suturing even normal tissue causes tissue injury and consequential inflammation and scarring due to the activation of relevant pathways [80]. Localizable drug delivery interventions could improve the surgical outcomes of implantable biomaterials and tissue engineered constructs. Interestingly, few researchers have considered using sutures themselves as drug delivery conduits. The majority of studies focused on covalently crosslinking [15, 81] or coating therapeutics [14, 16, 56] to existing sutures. However, these systems release drug in a matter of days and consequently limit the sustained release of therapeutics. To date, long-term drug delivery sutures are not commercially available. Ethicon is the only company currently manufacturing a line of Plus[®] sutures coated with a broad-spectrum antibacterial drug (triclosan) that elutes over a relatively short 7-day period [82]. One approach to achieve controlled, sustained delivery of drugs would be to impregnate or encapsulate therapeutics within the polymer matrix of wet spun fibers.

The overarching goal of this research is to develop drug-eluting sutures for the prevention of fibrocalcifications in tissue engineered cardiovascular constructs. This is particularly critical in cardiovascular anastomoses in young children where growth of a tissue engineered construct is desired [83, 84]. If the suture lines do not grow, then the limitation will be at the surgical sites of implantation within the bloodstream. The challenge with this work is to develop wet spun sutures that can withstand surgical implantation and deliver therapeutics over long periods of time (months). Dexamethasone, a potent anti-inflammatory drug, and noggin, a bone morphogenic protein antagonist, will be evaluated as putative targets to treat the fibrocalcific

response of sutured tissue engineered heart valves in an established *in vivo* ovine model for calcification.

1.6 References

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1.7 Figures

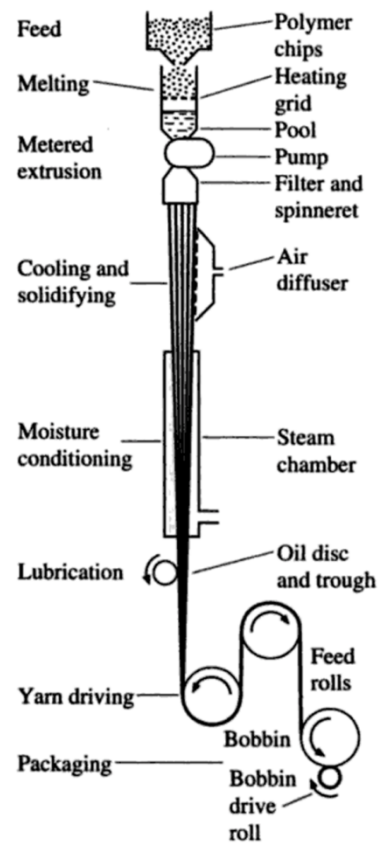


Figure 1.1: Schematic diagram of the melt spinning process. Thermoplastic polymers are melted and extruded through a spinneret to form monofilaments and then drawn through a series of up-take bobbins to impart strength. Adapted from [5].

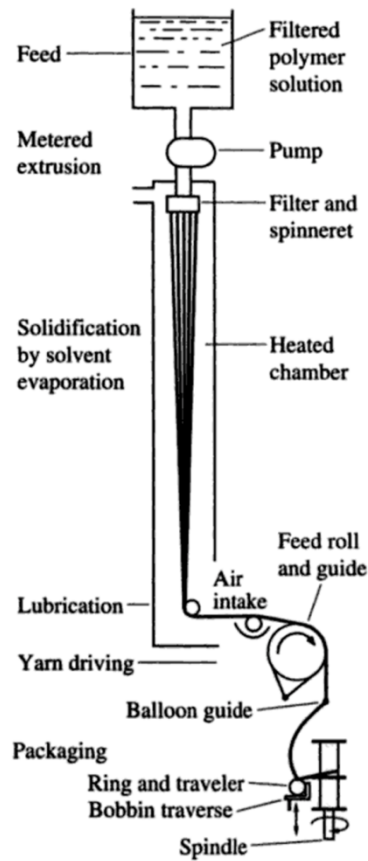


Figure 1.2: Schematic diagram of the dry spinning process. Polymers are dissolved in a volatile solvent and extruded through a spinneret to form monofilaments. Fibers are then drawn through a series of up-take bobbins to facilitate solvent evaporation and impart strength. Adapted from [5].

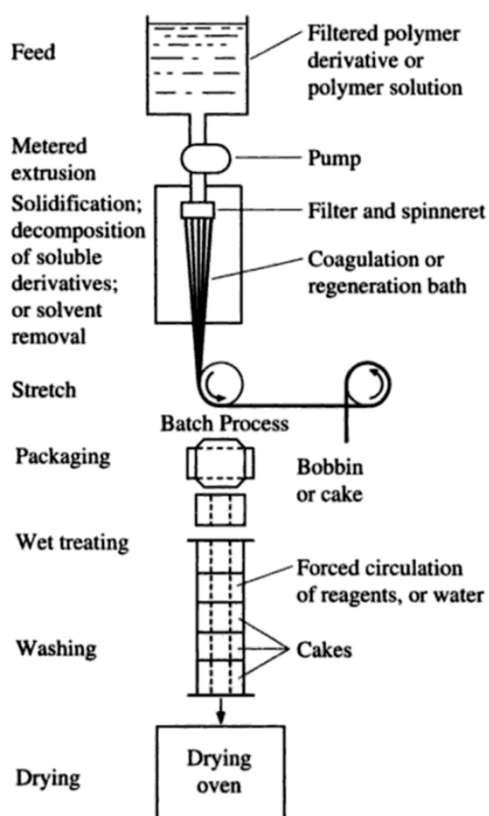


Figure 1.3: Schematic diagram of the wet spinning process. Polymers are dissolved in a solvent and extruded through a spinneret into a coagulation bath. Counter-diffusion of liquid polymer streams causes gelation and precipitation of the extrudate into monofilaments. Fibers are then drawn through a series of up-take bobbins to impart strength. Adapted from [5].

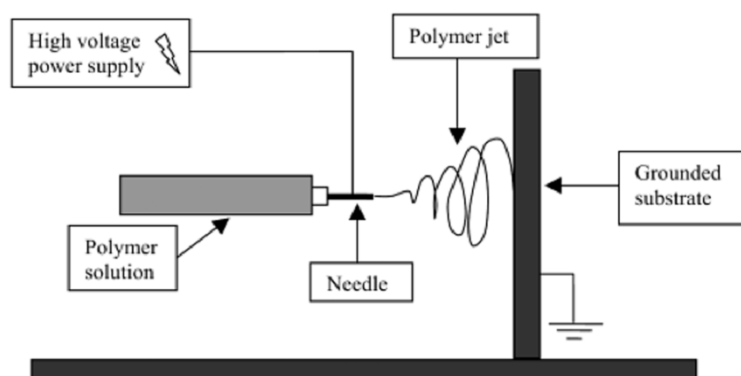


Figure 1.4: Schematic diagram of the electrospinning process. Polymers are dissolved in a solvent and propelled by electrostatic forces from a spinneret (syringe needle). Ultra-thin polymer streams are transformed into fibers as they are collected away from the spinneret on a grounded substrate [31].

Chapter 2

A novel wet extrusion technique to fabricate self-assembled microfiber scaffolds for controlled drug delivery

We have developed a novel wet extrusion process to fabricate nonwoven self-assembled microfiber scaffolds with uniform diameters less than 5 μm and without any post-manipulation. In this method, a poly(L-lactic acid) (PLLA) solution flows drop wise into a stirring nonsolvent bath, deforming into liquid polymer streams that self-assemble into a nonwoven microfiber scaffold. The ability to tune fiber diameter was achieved by decreasing polymer spin dope concentration and increasing the silicon oil to petroleum ether ratio of the nonsolvent spin bath. To demonstrate the drug delivery capabilities of scaffolds, heparin was encapsulated using a conventional water-in-oil (W/O) emulsion technique and a cryogenic emulsion technique developed in our lab. Spin dope preparation was found to significantly effect the release kinetics of self-assembled scaffolds by altering the interconnectivity of pores within the precipitating filaments. After 35 days, scaffolds prepared from W/O emulsions released up to 45% encapsulated heparin, whereas nearly 80% release of heparin was observed from cryogenic emulsion

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formulations. The versatility of our system, combined with the prolonged release of small molecules and the ability to control the homogeneity of self-assembling scaffolds, could be beneficial for many tissue regeneration and engineering applications.

2.1 Introduction

Since its conception, one of the primary goals of tissue engineering is to develop living functional tissue constructs that replace diseased or injured tissues and organs [1]. One prominent strategy is to seed appropriate cells *in vitro* on a biodegradable (synthetic or natural) scaffold to act as a morphological guide for tissue regeneration. However, for the repair and regeneration of complex tissues, a sequence of biological cues is often necessary. To this end, many drug delivery scaffolds have been designed for alternative tissue engineering strategies.

Fiber-based scaffolds offer advantages over other scaffold designs in that they possess high porosities with highly interconnected pore networks, have high surface area-to-volume ratios, and can be oriented and molded into different shapes and sizes [2]. To date, biodegradable fiber-based scaffolds have been fabricated by a number of methods, including melt spinning [3], solution (wet and dry) spinning [3-7], and electrospinning [8]. While there are numerous studies on fiber fabrication, only a small subset of this work focuses on the controlled release of biotherapeutics from fibers. Melt spinning is unsuitable for encapsulating proteins because the high processing temperatures can cause denaturation. Wet solution spinning is appealing for the encapsulation of water-soluble drugs since both the solvent and coagulant can be non-aqueous. The resulting fibers, however, are often too large (30-200 μm) for soft tissue engineering applications [5-7]. Electrospinning is a versatile method used to fabricate fibers on the nano- and micro-scale and is the most common method used to encapsulate therapeutics [9-13]. Asserting control of the initial burst, however, continues to be a difficult task due to the

high surface area-to-volume ratio of the nano-sized electrospun fibers and particularly for the encapsulation of water-soluble drugs [10, 12, 13].

This study introduces a new method for the fabrication of polymeric self-assembled scaffolds with controlled drug delivery capabilities. The purpose of this study was to evaluate our novel wet extrusion method to fabricate self-assembled scaffolds composed of poly(L-lactic acid) (PLLA) microfibers. The aims of this work were three-fold: (1) to control microfiber fabrication, (2) to define conditions to produce microfibers with a narrow diameter distribution, and (3) to encapsulate a model hydrophilic drug (heparin) and characterize release kinetics from self-assembled scaffolds.

2.2 Materials and methods

2.2.1 Materials

Poly (L-lactic acid) (PLLA, i.v. = 1.04 dL/g in CHCl_3) was purchased from Lactel Biodegradable Polymers (Birmingham, AL). Poly(D,L-lactic-co-glycolic acid) (PLGA) RG 503H (i.v. = 0.32-0.44 dL/g in CHCl_3) and RG 502H (i.v. = 0.16-0.24 dL/g in CHCl_3) were purchased from Boehringer Ingelheim (Petersburg, VA). Heparin sodium salt (170 UPS units/mg), Azure A dye, and sorbitan trioleate (Span 85, HLB 1.8), methylene blue, and β -carotene were all purchased from Sigma-Aldrich (St. Louis, MO) and used as supplied. Turmeric was purchased from Eli Lilly (Indianapolis, IN). Silicon oil (SO, 200 Fluid, 100 cSt) and dichloromethane (DCM) were purchased from Dow Corning Corporation (Midland, MI) and Burdick and Jackson (Muskegon, MI), respectively. Reagent grade petroleum ether (PE) was obtained from Mallinckrodt AR (Paris, Kentucky).

2.2.2 Fabrication of PLGA micro/nanospheres

Micro/nanospheres were fabricated by solvent removal[14] and phase-inversion nanoencapsulation (PIN)[15] methods. Blank PLGA (RG 502H) microspheres were fabricated by the solvent removal method to demonstrate the effect of nonsolvent bath composition on the formation of microsphere and fiber mixtures. In brief, 250 mg of PLGA (RG 502H) was dissolved in 5 mL DCM. 125 μ L of ultra-pure water and 100 μ L was added to the polymer / solvent mixture and vortexed for one minute to create a stable emulsion. The solution was then poured into a 500 mL nonsolvent bath of 1:1 PE to SO and 100% SO stirring at 1,300 rpm. After 2.5 min, the entire mixture was added to an excess of PE and allowed to mix for 30 min. Samples were filtered and washed with additional PE. To demonstrate the ability to incorporate drug-loaded nanospheres during the self-assembly of PLLA scaffolds, PLGA (RG 503H) nanospheres were fabricated by PIN. In this method, β -carotene was used as a model therapeutic for demonstration purposes only. 250 mg of PLGA (RG 503H) and 2 mg of β -carotene were dissolved in 2.5 mL of DCM. The mixture was then poured into 500 mL of PE, resulting in spontaneous phase-inversion and subsequent formation of nanospheres encapsulating drug. 250 mL of the PIN nanosphere suspension was added to 125 mL of SO and stirred at 500 rpm for 3 min to stabilize the mixture prior to microfiber scaffold self-assembly described below.

2.2.3 Fabrication of self-assembled microfiber scaffolds

Self-assembled polymer scaffolds were fabricated from 2.5, 5, 7.5, and 10% (w/v) PLLA spin dope solutions. PLLA was dissolved in 2.5 mL DCM and poured into a 5 mL glass syringe equipped with a 17-gauge spinneret. The spin dope solution flowed gravimetrically through the spinneret, which was positioned close to the edge of a 400 mL beaker directly above a 375 mL nonsolvent coagulation bath stirring at 500 rpm. The spin dope solution was extruded as droplets that upon contact with the nonsolvent

bath, deformed into micron-sized fibers due to the shear forces generated by the rotating shaft / impeller in the spin bath. The microfibers migrated towards the Teflon coated impeller shaft (6 mm diameter) forming self-assembled scaffolds (**Figure 2.1**). All of the spin dope solutions were extruded in less than one minute. Scaffolds were left in the stirring spin bath for a residence time of 5 minutes, and then removed from the impeller shaft and washed in excess petroleum ether. The effect of nonsolvent composition was studied by varying the ratio of petroleum ether to silicon oil. It was possible to fabricate scaffolds containing methylene blue and turmeric by adding dyes directly to the spin dope solution prior to wet spinning.

2.2.4 Fabrication of drug-eluting self-assembled microfiber scaffolds

Drug-loaded spin dope solutions were prepared using two techniques. In the first technique, heparin was dissolved in ultra-pure water at 3.4, 5.1, and 12.8 mg mL⁻¹. 125 μ L of the aqueous heparin solution was then added to 2.5 mL of PLLA in DCM (100 mg mL⁻¹) for an aqueous to organic phase ratio of 1:20. A stable water-in-oil (W/O) emulsion was formed by adding 100 μ L of Span 85 followed by vortexing for 60 seconds. The emulsion was immediately poured into the spinneret for the formation of microfiber scaffolds as previously described. This technique is referred to hereafter as ‘W/O emulsion’.

In the second technique, heparin was micro / nanonized using a cryogenic emulsion method developed in our lab for the encapsulation of therapeutics within polymeric microspheres [16]. Briefly, 0.5 mL heparin solutions were emulsified in 10 mL of PLLA (25 mg mL⁻¹) in DCM and 100 mL of Span 85 by vortexing for 60 seconds. The W/O emulsion was immediately frozen in liquid nitrogen and lyophilized for 48 hours. Following lyophilization, micro / nanonized heparin was distributed in a continuous phase of polymer. The dried polymer and heparin product was then reconstituted in 2.5 mL DCM at a concentration of 100 mg mL⁻¹. The suspension of solubilized polymer and

insoluble micro / nanonized heparin particles was then poured into the glass syringe and wet spun as described above. This technique is referred to hereafter as ‘cryogenic emulsion’. All heparin-loaded microfiber scaffolds were prepared using 10% (w/v) polymer concentration, 2:3 PE to SO nonsolvent bath ratio, rotational bath speed of 500 rpm, and residence time of 5 minutes. Blank microfibers were also fabricated using W/O emulsion and cryogenic emulsion techniques.

2.2.5 Scanning electron microscopy (SEM) analysis

Lyophilized microfibers were mounted on adhesive metal stubs and sputter-coated with a layer of 50–100 Å layer of gold-palladium (Emitech, Kent England). Samples were viewed and digital images were taken with a Hitachi S-2700 scanning electron microscope (Tokyo, Japan) using Quartz PCI software.

2.2.6 Determination of microfiber diameter

Microfiber diameters were measured from scanning electron micrographs taken at a magnification of 300x. For each formulation, fiber diameters from ten fields of view were measured using Quartz PCI v.2.1 software (Vancouver, BC, Canada). The diameters of scaffolds fabricated with 2.5% (w/v) polymer concentration and all batches fabricated with 100% petroleum ether were not determined because of the large prevalence of beaded, bulging, and branched fibers. Irregular-shaped fibers were rarely observed in 5%, 7.5%, and 10% (w/v) PLLA formulations; misshapen fibers were excluded in diameter measurements. At least 150 fiber diameters were measured for each formulation. The diameters of self-assembled microfiber scaffolds were compared in SPSS v.19 (Chicago, IL) using a nonparametric Kruskal-Wallis test. *Post hoc* analyses for multiple comparisons were carried out using the Mann-Whitney tests with Bonferroni correction ($\alpha = 0.01$). A *p* value of less than 0.05 was considered statistically

significant.

2.2.7 Differential scanning calorimetry (DSC) analysis

Scaffolds (2-4 mg) were analyzed for thermal transitions using a DSC-7 (Perkin Elmer) equipped with an Intracooler 2 intercooling system. DSC conditions were set to include the glass transition temperature and melting temperature of PLLA, with heating scans at least 20°C above and below thermal transitions. Scaffold samples were subjected to: 1) cooling to -25°C, 2) heating to 250°C at 10°C/min, 3) cooling to -25°C at 10°C/min, and 4) reheating the sample to the upper limit again at the initial rate. The percent crystallinity (X_c) of the PLLA microfibers was calculated using the following equation:

$$X_c = \frac{\Delta H_m}{93.7} \times 100 \quad (\text{equation 2.1})$$

where ΔH_m is the enthalpy of melting of the samples and 93.7 J/g is specific heat of melting of a 100% crystalline PLLA as reported in literature [17].

2.2.8 Determination of encapsulation efficiency

Triplicate samples (10-15 mg) of each formulation were dissolved in 1 mL of DCM in glass scintillation vials. 2.0 mL of PBS buffer was added to the organic solution. The mixture was placed on a rotating tumbler for 3 hours to facilitate heparin extraction. 0.5 mL of the aqueous phase was then transferred to a microcentrifuge tube and centrifuged for 5 minutes. The 0.5 mL aliquot was then assayed for heparin concentration as using a modified Azure A assay [18]. In brief, 20 µL of unknown samples were added in triplicate to 96-well plates followed by 150 µL of a 7.5% Azure A solution. Samples and standards were read by UV absorption at 500 nm using a

Spectramax 384 Plus UV spectrophotometer (Molecular Devices, Sunnyvale, CA) to determine heparin concentration.

2.2.9 *In vitro* heparin release study

Triplicate scaffold samples (10-15 mg) from each formulation were suspended in 1 mL of PBS buffer and incubated at 37 °C. At pre-determined time points, 850 µL of releasate was extracted and replaced with fresh PBS buffer at the same volume. Releasates were stored at -20 °C until further analysis. Release kinetics from microfiber scaffolds was determined using the Azure A assay described above in the determination of encapsulation efficiency. The UV absorbance of heparin releasates was measured at 500 nm and concentrations calculated against a standard curve. Percent cumulative release of heparin was normalized to the actual amount encapsulated.

2.3 Results and discussion

2.3.1 Effect of processing conditions on microfiber scaffold formation

The experimental conditions for fabricating polymeric microspheres by solvent removal have been well characterized [14, 19]. Polymer concentration, stirring rate, and phase separation parameters dictate microsphere formation, diameter, and morphology. In our lab, we have used the solvent removal technique for the encapsulation of a multitude of therapeutics within a variety of polymeric microspheres [20-23]. We have observed that when we increase the nonsolvent viscosity in this system keeping the same stirring rate, both microspheres and fibers are formed. **Figure 2.2** illustrates the effect of nonsolvent composition on the morphology of PLGA (RG 502H) microspheres. PLGA (RG 502H) microspheres are formed using 1:1 petroleum ether to silicon oil nonsolvent bath

(**Figure 2.2A**), whereas fibers and microspheres are formed using 100% silicon oil (**Figure 2.2B**). In both cases the stirring rate of 1,300 rpm and spin dope solution to nonsolvent ratio of 1:100 was kept the same for both systems. In this paper, we systematically aimed to optimize the conditions under which homogeneous microfibers are formed. This new method produces micron-sized fibers through the hybridization of conventional microsphere and wet solution spinning techniques.

In conventional wet solution spinning, a polymer spin dope solution flow rate is typically controlled such that there is only a single filament (30-200 μm) being formed. The fiber is then wound by a rotating mandrel with adjustable speed to modulate fiber diameter, which is principally controlled by equipment parameters, such as spinneret geometry and draw ratio [5, 6]. In our method, the spin dope solution is introduced drop wise into a stirring nonsolvent bath causing it to elongate and shear into thin liquid streams upon contact. Counter-diffusion of solvent and nonsolvent commences instantly and the polymer streams precipitate into microfilaments when the solution becomes thermodynamically unstable. Microfilaments (4-15 μm) migrate through the nonsolvent bath towards a Teflon coated shaft / impeller (centered in the bath) and wrap around it forming a nonwoven self-assembled microfiber scaffold (**Figures. 2.1B, 2.3A**).

To demonstrate the versatility of this method, various dyes were encapsulated into PLLA microfibers for visual effect. **Figure 2.3B** shows an image of a cross-sectioned 10% (w/v) PLLA scaffold fabricated from a mixture of methylene blue / turmeric (green), methylene blue (purple), and dye-free (white) spin dope solutions. This scaffold was created from three separate 2 mL spin dope solutions sequentially added to a stirring nonsolvent bath of 2:3 petroleum ether (PE) to silicon oil. (SO). The colored microfibers illustrate the ability for creating spatially distinct scaffolds. The spatial distribution and composition of drug delivery microfibers within the scaffold would be beneficial for many tissue engineering applications. Modulation of therapeutics could also be achieved by incorporating nanospheres within the scaffold during self-assembly.

For example, nanospheres that contained β -carotene (just for demonstration) were integrated into scaffolds by adding 250 mL of a PLGA (RG 503H) phase-inverted nanosphere (PIN) suspension in PE to 125 mL SO prior to introducing a PLLA spin dope solution to the stirring nonsolvent bath. **Figure 2.3C** shows β -carotene-loaded PLGA (RG 503H) nanospheres fused to 10% (w/v) PLLA scaffold microfibers.

Other research groups have used a stirring nonsolvent bath termed ‘hydro-spinning’ for the formation of alginate / chitosan scaffolds [24, 25]. In contrast to our extrusion technique, fibers produced from the hydro-spinning technique formed irregular ribbon-like structures upon extrusion of spin dope solutions by a pump-controlled syringe. Moreover, hydro-spun filaments required further manipulation for scaffold formation similar to conventional wet spinning techniques and were only evaluated as morphological guides for tissue engineering applications.

2.3.2 Effect of polymer concentration and nonsolvent composition on microfiber morphology

Scaffolds were fabricated from spin dope solutions of PLLA dissolved in DCM at 2.5, 5, 7.5, and 10% (w/v) to investigate the effects of polymer concentration and nonsolvent composition on microfiber formation. Independent of polymer concentration, thin sheets formed with 100% petroleum ether (**Figure 2.4, Row A**), which resulted in scaffolds with weak structural integrity and cloudy nonsolvent baths with floating polymer fragments. Scaffold integrity improved and morphology changed dramatically with nonsolvent baths composed of silicon oil and petroleum ether. Great heterogeneity in morphology (i.e. microspheres; elongated beads; flat, segmented ribbons; large globular structures with multiple branches; kinked microfibers; and thin sheets) was observed for all scaffolds made with 2.5% (w/v) polymer concentrations (**Figure 2.4, Column I**). Scaffold morphology became more fibrous in nature with 5% and 7.5% (w/v) polymer concentration and with increasing silicone oil content within the nonsolvent spin bath

(**Figure 2.4, Column II, III**). Scaffolds were composed of smooth fibers with 10% polymer concentration at all nonsolvent compositions, with the exception of 100% PE (**Figure 2.4, Column IV**).

We believe the solubility parameters of solvent ($\delta_{\text{DCM}} = 20.2 \text{ MPa}^{1/2}$) and nonsolvent ($\delta_{\text{PE}} = 14.8 \text{ MPa}^{1/2}$) significantly influence microfiber geometry. For all polymer concentrations, rapid precipitation occurred with 100% PE, leading to fiber malformation. However, the addition of silicon oil ($\delta_{\text{SO}} = 16.6 \text{ MPa}^{1/2}$) resulted in the formation of more homogeneous microfibers with circular geometry. The difference in the solubility parameter between DCM and PE and DCM and SO is 5.4 units and 3.6 units, respectively, making PE a better nonsolvent for the PLLA microfiber than SO. Thus, slower precipitation occurs with SO, allowing more time for the polymer droplet to elongate and thin into fibrous strands.

2.3.3 Effect of polymer concentration and nonsolvent composition on microfiber diameter

The diameters of microfiber scaffolds produced from 5%, 7.5%, and 10% (w/v) PLLA concentrations with varying nonsolvent composition were measured to study the effect of fabrication variables on fiber distribution. The diameters of scaffolds fabricated with 2.5% (w/v) polymer concentration and all batches fabricated with 100% petroleum ether were not determined due to the large prevalence of fiber malformation. As shown in **Figure 2.5**, varying spin dope concentration and nonsolvent composition can produce microfibers with diameters ranging from 400 nm to 45 μm . In general, 5% and 7.5% (w/v) polymer concentrations lead to scaffolds with significantly smaller fiber diameters ($p < 0.05$) than scaffolds fabricated with 10% (w/v) polymer concentration. This trend is consistent across all nonsolvent compositions. The nonsolvent bath composition substantially altered fiber diameter distributions. By increasing the silicon oil content from 20% to 100%, the fiber diameter (mean $\pm$ SD) dropped from 8.7 ± 6.8

μm to $4.1 \pm 3.8 \mu\text{m}$ for 5% scaffolds, from $11.5 \pm 11.9 \mu\text{m}$ to $4.1 \pm 3.0 \mu\text{m}$ for 7.5% scaffolds, and from $14.9 \pm 10.2 \mu\text{m}$ to $12.1 \pm 7.1 \mu\text{m}$ for 10% (w/v) scaffolds (**Figure 2.5A-C**). The effect of silicon oil content on the microfiber diameter is less pronounced with scaffolds produced from 10% (w/v) polymer concentrations. However, a decrease in the microfiber variability is still observed with increasing silicon oil content up to 60%. Increasing the silicon oil content of the nonsolvent bath beyond this did not result in further reduction in mean fiber diameter or narrowing of diameter distribution.

Given these findings, we believe fiber diameter is predominantly influenced by nonsolvent solution viscosity and polymer concentration. The kinematic viscosity of SO and PE is 100 cSt and ~ 31 cSt, respectively. The addition of SO increases the viscosity of the spin bath, thus increasing the shear forces on the polymer droplet as it migrates towards the shaft / impeller. These forces have a greater impact on low polymer concentration solutions, since they deform more readily under shear stresses. This may also cause the liquid polymer droplet to elongate and break into multiple smaller strands before assembling on the rotating shaft / impeller.

2.3.4 Effect of heparin loading on microfiber morphology

Heparin was encapsulated into PLLA microfibers to demonstrate the potential of self-assembled scaffolds for use as controlled release systems. All scaffolds were fabricated using a 10% (w/v) PLLA concentration and 60% silicon oil nonsolvent bath composition. Microfibers prepared using the W/O emulsion method exhibited globular structures with large bulges (**Figure 2.6, Row A**). Large voids were also present in cross-sectioned microfibers (**Figure 2.6, Row B**). Other researchers have observed similar morphologies in the wet spinning of W/O emulsions. Rissanen et al. attributed irregular structures and voids observed with wet spun poly(L,D-lactic acid) fibers to the formation of air bubbles during the formation of the primary emulsion and rapid phase separation [26]. No irregularities were found with microfibers prepared using the

cryogenic emulsion technique. Since the aqueous phase of the primary emulsion is removed by the cryogenic emulsion method, it is likely that the irregularities observed with scaffolds prepared from W/O emulsion are due to air bubbles and rapid precipitation. Thus, spin dope solutions prepared by cryogenic emulsion would be optimal for the fabrication of homogeneous self-assembled microfiber scaffolds.

2.3.5 Effect of polymer concentration and nonsolvent composition on the thermal properties of microfibers

Differential scanning calorimetry (DSC) was used to investigate the effects of polymer concentration and nonsolvent composition on glass transition temperature (T_g), melting temperature (T_m), enthalpy of melting (ΔH_m), and percent crystallinity (X_c) of microfiber scaffolds. A summary of the results is shown in **Table 1**. The T_g , T_m , and of X_c of unprocessed PLLA were 53.7°C, 173.7°C, and 53.1%, respectively. Polymer concentration and nonsolvent composition did not appear to have an effect on T_m . With 100% PE and 100% SO, the T_g remained stable across batches made with different polymer concentrations. However, with SO and PE nonsolvent mixtures, the T_g of microfibers made with 10% (w/v) polymer concentration decreased from 2.5%, 5%, and 7.5% (w/v) formulations. The T_g of 7.5% and 10% (w/v) polymer concentrations also decreased with increasing silicon oil content (with the exception of 100% silicon oil). The decrease in T_g observed with 7.5% and 10% (w/v) polymer concentrations may be attributed to residual silicon oil trapped within the microfibers, a problem exacerbated by the use of high polymer concentrations and higher silicon oil content in the nonsolvent bath. At 100% silicon oil, it is possible that the counter-diffusion of solvent and nonsolvent is slowed enough that shear stresses begin to increase T_g by elongation / orientation of amorphous polymer chains. Since there is also more time for counter-diffusion of solvent and nonsolvent, this could also result in less entrapment of silicon oil within the fibers during gelation.

Interestingly, all scaffolds fabricated using 100% PE had increased crystallinity in comparison to raw polymer. As the silicon oil content increased, there was also a slight decrease in crystallinity with 2.5% (w/v) polymer concentration. In wet spinning, phase separation of the polymer solution is initiated into two phases, a polymer lean (porous fiber structure) and polymer rich (polymer matrix) phase. The polymer lean phase gains mass while the polymer rich phase becomes more concentrated until gelation occurs. Counter-diffusion of solvent and nonsolvent and phase separation of polymers take place during residence time, or the amount of time the fiber is left in the coagulation bath. In our system, the addition of silicon oil slows the phase separation of the liquid polymer streams as they deform in the spin bath. Spin dope surface coagulation occurs more rapidly in high polymer concentrations, resulting in a fiber with ‘skin-core’ structure. This dense, non-porous ‘skin’ could have served as a diffusional barrier for solvent and nonsolvent. Skin thickness decreases with decreasing spin dope concentration.

2.3.6 Effect of drug loading and spin dope preparation on the thermal properties of microfibers

The thermal properties of drug-loaded and blank microfibers were studied using DSC to determine the effects of drug encapsulation and spin dope preparation. Calculations from the thermal transitions of the first heating scan are shown in **Table 2**. No significant differences in T_g were observed between heparin-loaded fibers in comparison to control fibers without drug. This could mean that heparin and the surfactant do not act as plasticizers and interact with the polymer matrix, or that there is too little drug or surfactant to impact the glass transition temperatures. The T_m and percent crystallinity of blank and heparin-loaded fibers prepared by W/O emulsion and cryogenic emulsion techniques was also consistent among batches. T_m and percent crystallinity of heparin-loaded and blank microfibers were consistent with 10% (w/v)

polymer scaffolds prepared without surfactant using 2:3 PE to SO nonsolvent composition (**Table 1**).

2.3.7 Microfiber encapsulation efficiencies

Actual loading and heparin encapsulation efficiencies are listed in **Table 3**. In general, formulations prepared using W/O emulsions had higher encapsulation efficiencies ranging from 53% to 87% in comparison to formulations prepared using cryogenic emulsions that had efficiencies ranging from 38% to 73%. Heparin loss from the cryogenic emulsion process can be attributed to heparin adsorption during the micro / nanonization process. While cryogenic emulsion formulations underwent similar heparin loss, more loss occurred with batches of lower theoretical loading. Heparin loss could be reduced if batch sizes were scaled up. Heparin loss from W/O emulsion formulations could have occurred during the rapid phase separation of coagulating microfibers. Although some heparin loss occurred during the self-assembly of microfiber scaffolds, the encapsulation efficiencies achieved in this work were greater than those achieved for the encapsulation of heparin within polymeric microspheres using W/O/W double emulsion solvent evaporation methods [27, 28]. The encapsulation process of our system occurs in a hydrophobic environment. Thus, our novel wet extrusion technique improves the encapsulation of small hydrophilic molecules, such as heparin, within hydrophobic polymers.

2.3.8 *In vitro* release kinetics of self-assembled scaffolds

Cumulative heparin release (expressed as percentage of actual loading) profiles from PLLA microfiber scaffolds are plotted in **Figure 2.7**. Compared to cryogenic emulsion formulations, the release of heparin from W/O emulsion scaffolds was significantly slower, with only 23-45% occurring within 35 days. Nearly 80% release of heparin was

observed from cryogenic emulsion formulations over the same time period. These findings can partly be explained by the morphology observed with SEM. We believe that the voids within W/O emulsion scaffolds had significantly reduced porous interconnectivity in comparison to cryogenic emulsion scaffolds. The lack of interconnectivity between pores prolongs the release of heparin; otherwise we would have seen almost immediate release of this small hydrophilic molecule. One explanation for reduced porous interconnectivity could be the 1:20 ratio between the aqueous solution and the polymeric solution used during fabrication. Chen et al. showed increasing in the W/O phase ratio from 1:60 to 1:24 substantially reduced the burst release of bovine serum albumin from PLGA microspheres [29]. In general, increasing the oil-to-water phase ratio reduces the effectiveness of the surfactant and decreases the stability of the emulsion. The fusion of water droplets during fabrication could lead to an increase in porous interconnectivity, thus reducing the tortuosity of drug molecules during diffusion and increasing initial burst release. The micro / nanonization of heparin particles during the cryogenic emulsion process could have also resulted in a more even distribution of heparin within microfibers, thus enhancing drug release. Heparin release among the same spin dope preparation occurred at similar rates despite increased drug loading with the exception of 1% (w/w) theoretical loading for scaffolds prepared using the W/O emulsion method. The daily release rates after the initial burst of heparin ranged from $0.1 \mu\text{g mg}^{-1}$ to $0.7 \mu\text{g mg}^{-1} \text{ day}^{-1}$ for W/O emulsion formulations, and from $0.5 \mu\text{g mg}^{-1}$ to $0.8 \mu\text{g mg}^{-1} \text{ day}^{-1}$ for cryogenic emulsion formulations.

The release of heparin from our system could be tailored by changing the polymer concentration and nonsolvent bath conditions to reduce the fibers diameters for enhanced release or by wet spinning higher molecular weight polymers for extended release. The release kinetics of W/O emulsion scaffolds from our study could also be increased using more dilute aqueous to organic phase ratios. Control over the release of small hydrophilic molecules, including heparin, would be beneficial for many applications in drug delivery and tissue regeneration. For example, the controlled

delivery of heparin from grafted blood vessels has been shown to inhibit vascular smooth muscle cell proliferation [30]. Heparin has also been used to improve the release kinetics and biological activity of heparin-binding growth factors, such as basic fibroblast growth factor (bFGF) [31].

2.4 Conclusions

We report, for the first time, a wet extrusion technique for the fabrication of self-assembled polymeric scaffolds with mean fiber diameters as small as 5 μm . The most striking advantage of this novel wet extrusion method over other wet spinning techniques is the one-step formation of nonwoven self-assembled microfiber scaffolds with tunable fiber morphology and drug release kinetics. Moreover, our system can be used for the simultaneous delivery of a multitude of therapeutics. These self-assembled scaffolds can be used as a platform for future studies in the regeneration of tissues that require a complex sequence of biological cues and structural support.

2.5 References

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2.6 Tables

Table 2.1: Summary of the thermal properties from the first DSC heating scan of self-assembled PLLA microfiber scaffolds

PE to SO ratio	T_g (°C)	T_m (°C)	ΔH_m (J/g)	X_c (%)
<i>2.5% PLLA</i>				
100% PE	60.6	172.7	55.3	59.0
4:1	62.9	173.0	56.2	60.0
3:2	61.9	173.7	53.6	57.2
2:3	61.9	172.9	52.2	55.7
1:4	61.3	174.0	48.6	51.9
100% SO	61.0	173.0	49.5	52.8
<i>5% PLLA</i>				
100% PE	61.0	172.5	53.0	56.6
4:1	63.6	173.5	53.6	57.2
3:2	56.7	172.4	51.2	54.6
2:3	60.2	172.7	52.3	55.8
1:4	61.0	172.7	51.2	54.6
100% SO	61.2	173.2	50.9	54.3
<i>7.5% PLLA</i>				
100% PE	62.2	172.5	55.8	59.6
4:1	62.8	173.2	54.4	58.1
3:2	61.3	173.7	52.9	56.5
2:3	57.3	172.7	51.9	55.4
1:4	56.9	172.0	54.4	58.1
100% SO	60.5	172.3	53.6	57.2
<i>10% PLLA</i>				
100% PE	60.1	173.3	54.3	58.0
4:1	53.2	174.5	53.2	56.8
3:2	53.8	173.7	51.9	55.4
2:3	47.6	173.7	53.6	57.2
1:4	49.1	172.4	56.4	60.2
100% SO	59.2	173.0	51.9	55.4
<i>Unprocessed PLLA</i>				
—	53.7	173.7	49.8	53.1

T_g , glass transition temperature

T_m , melting temperature

ΔH_m , enthalpy of the melting endotherm

X_c , percent crystallinity

Table 2.2: Summary of the thermal properties from the first DSC heating scan of blank and heparin-loaded 10% (w/v) self-assembled microfiber scaffolds

Microfiber formulation	T_g (°C)	T_m (°C)	ΔH_m (J/g)	X_c (%)
<i>W/O emulsion</i>				
Blank	55.1	174.0	51.3	54.8
0.67%	57.3	173.5	50.3	53.7
1.0%	55.6	173.7	52.2	55.7
2.5%	56.2	173.9	51.5	55.0
<i>Cryogenic emulsion</i>				
Blank	56.6	172.8	51.9	55.3
0.67%	57.5	175.0	53.7	57.3
1.0%	56.1	173.7	53.0	56.5
2.5%	57.5	173.7	52.1	55.6

T_g , glass transition temperature

T_m , melting temperature

ΔH_m , enthalpy of the melting endotherm

X_c , percent crystallinity

Table 2.3: Encapsulation efficiency of self-assembled microfiber scaffolds

Heparin loading of dope solution (%w/w)	Heparin loading of self- assembled scaffold (%w/w)	Heparin encapsulation efficiency (%)
<i>W/O emulsion</i>		
0.67	0.35	52.8 ± 0.0
1.0	0.87	86.7 ± 0.1
2.5	1.66	66.4 ± 0.0
<i>Cryogenic emulsion</i>		
0.67	0.25	37.7 ± 0.1
1.0	0.50	50.3 ± 0.1
2.5	1.82	72.8 ± 0.1

2.7 Figures

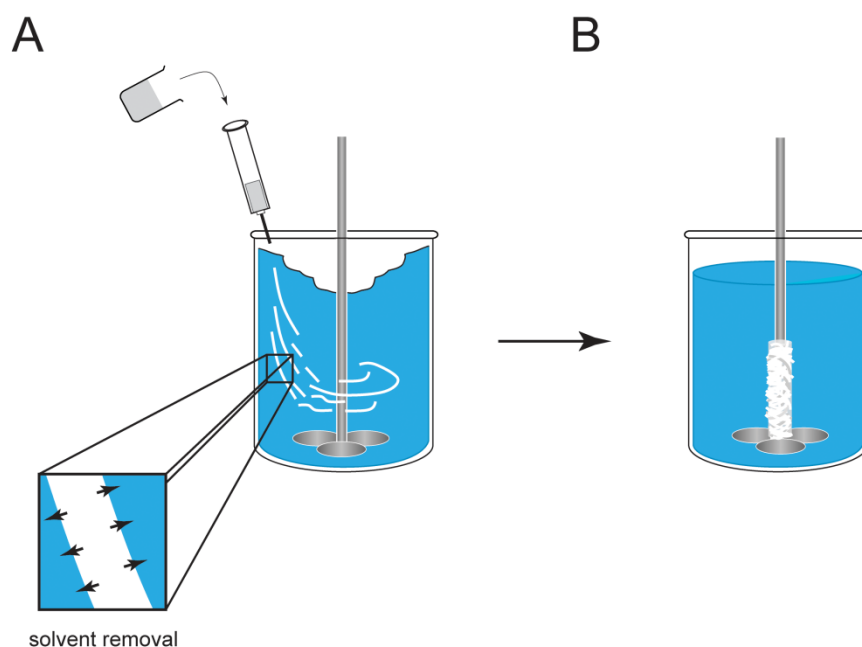


Figure 2.1: Schematic of the wet extrusion process used to fabricate self-assembled microfiber scaffolds. (A) The spin dope solution is introduced drop wise into a stirring nonsolvent bath causing each droplet to elongate and shear into thin strands upon contact; (insert) direction of solvent diffusion from spin dope solution into the nonsolvent bath. As the strands migrate towards the center of the bath, they are drawn further until (B) reaching the shaft / impeller forming a nonwoven, self-assembled scaffold.

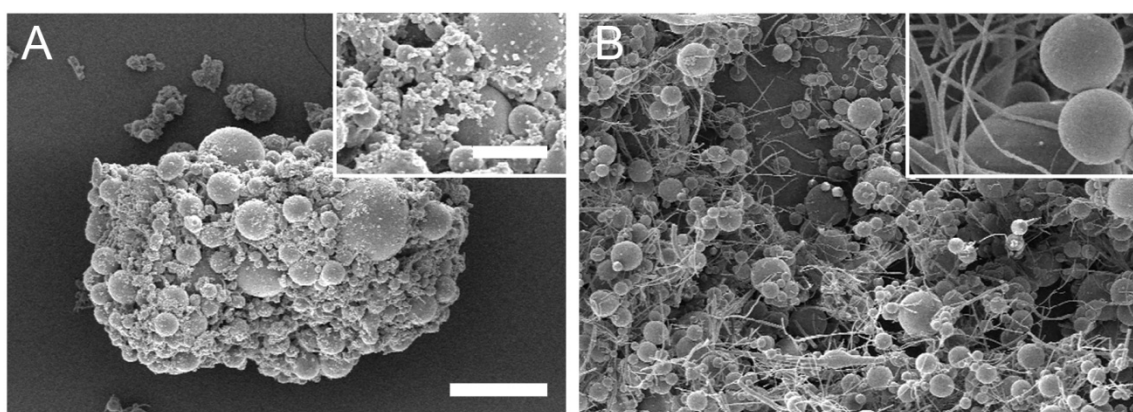


Figure 2.2: Scanning electron micrographs of 5% (w/v) PLGA (RG 502H) microspheres prepared with (A) 1:1 petroleum ether (PE) to silicon oil (SO) and (B) 100% SO nonsolvent composition. The fabrication parameters were a 500 mL nonsolvent bath, a residence time of 30 minutes, and a stirring rate of 1,300 rpm. Scale bars, (A, B) 100 μm (200x magnification) and (insets) 15 μm (1.0kx magnification).

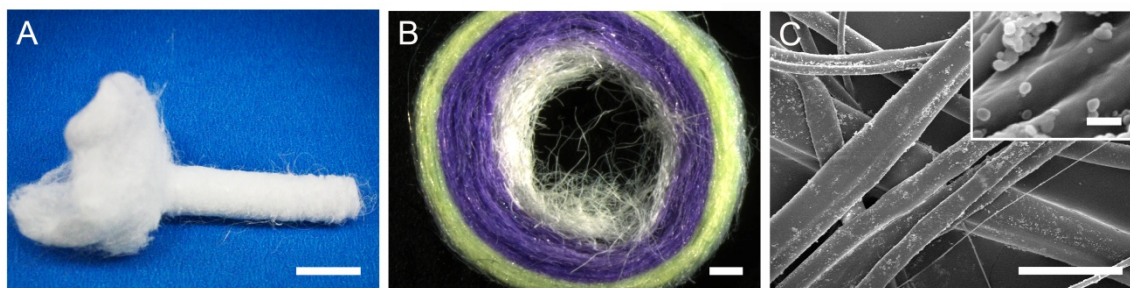


Figure 2.3: The wet extrusion process is a versatile method to create self-assembled microfiber scaffolds. (A) Digital image of a typical PLLA microfiber construct after removal from the Teflon coated mandrel. (B) Digital image of a cross-sectioned scaffold with microfibers loaded with dyes demonstrating ability for creating spatially distinct scaffolds. (C) Scanning electron micrograph of drug-loaded PLGA (RG 503H) nanospheres prepared by phase-inversion nanoencapsulation (PIN) fused to 10% (w/v) PLLA scaffold microfibers. Scale bars, (A) 10 mm, (B) 1 mm, (C) 100 μm (300x magnification) and (inset) 1 μm (10kx magnification).

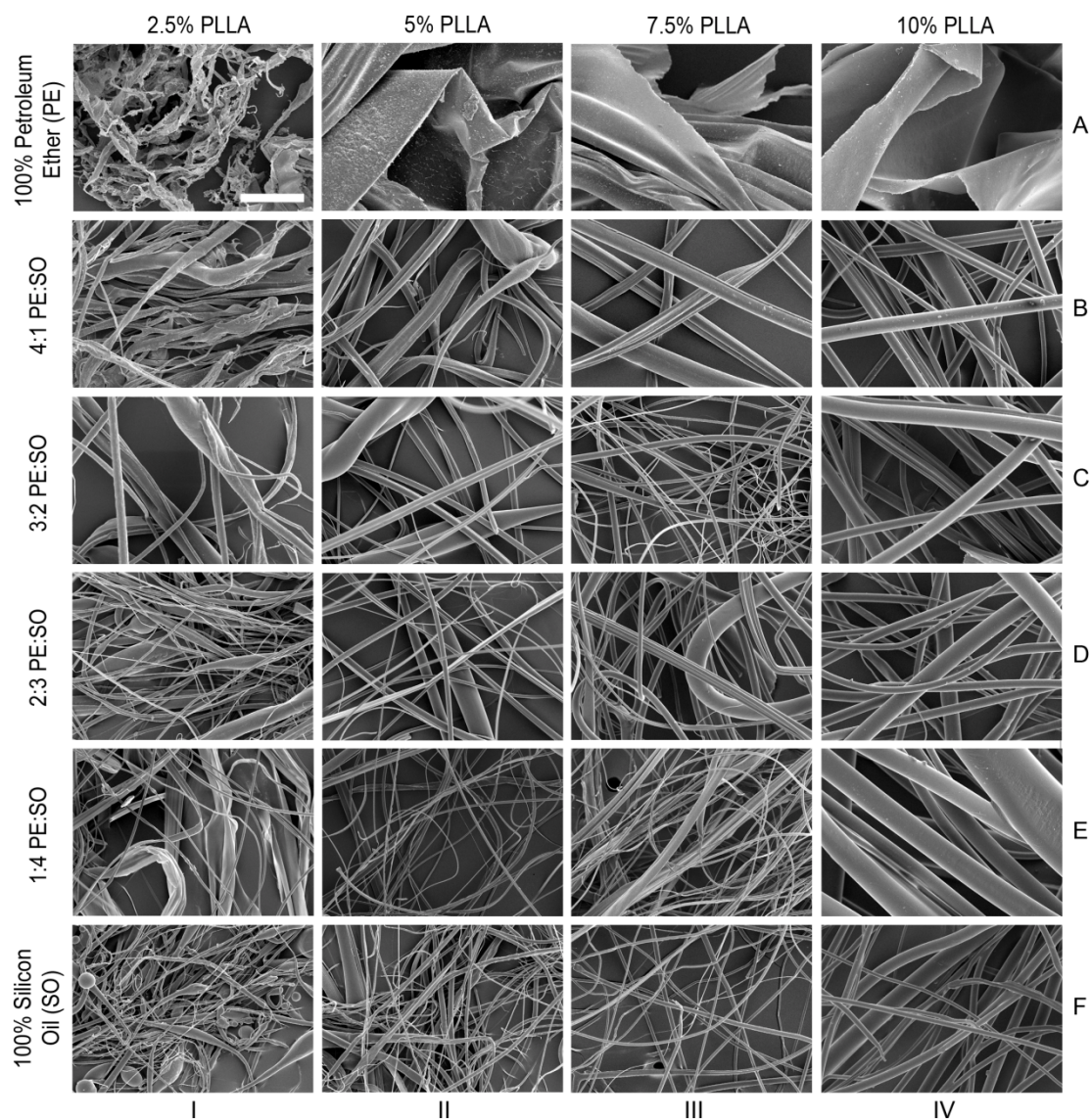


Figure 2.4: Scanning electron micrographs of PLLA microfibers prepared with 2.5%, 5%, 7.5%, and 10% (w/v) spin dope solutions and varying petroleum ether (PE) and silicon oil (SO) nonsolvent compositions. Group identifiers are shown in the top and left margins. Column and row identifiers are shown in the bottom and right margins. Scale bar, 100 μm (300x magnification).

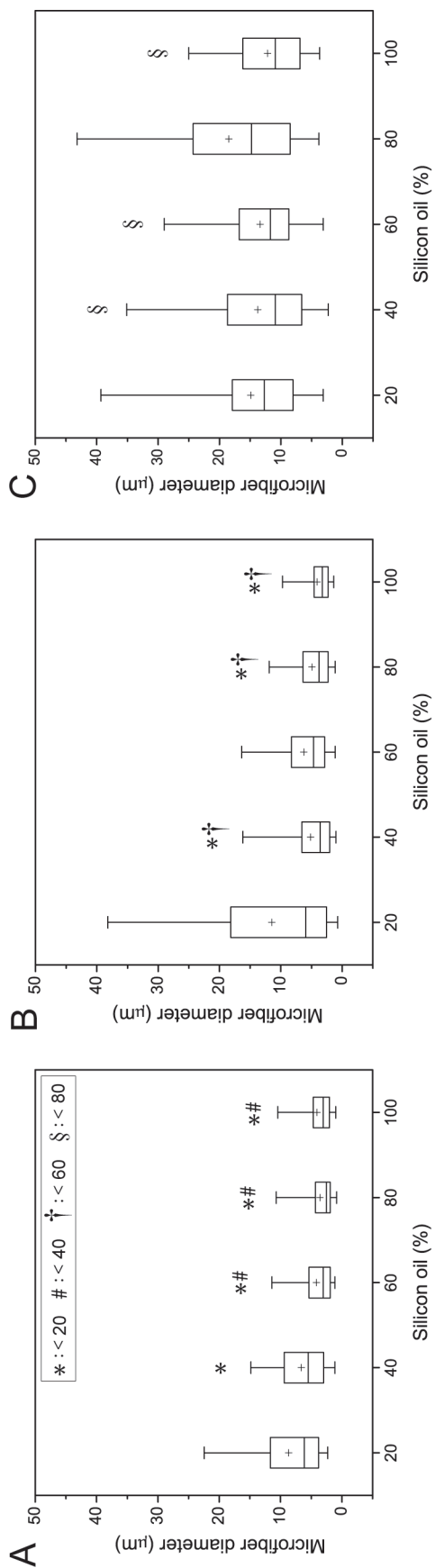


Figure 2.5: Microfiber diameter box plot distributions of self-assembled PLLA scaffolds fabricated from (A) 5%, (B) 7.5%, and (C) 10% (w/v) spin dope solutions. ‘+’ indicates mean microfiber diameter, tops and bottoms of boxes indicate interquartile ranges, bands indicate medians, and whiskers extend from 5th to 95th percentiles. *, #, †, §: significant in Kruskal-Wallis test with *post hoc* Mann-Whitney tests with Bonferroni correction.

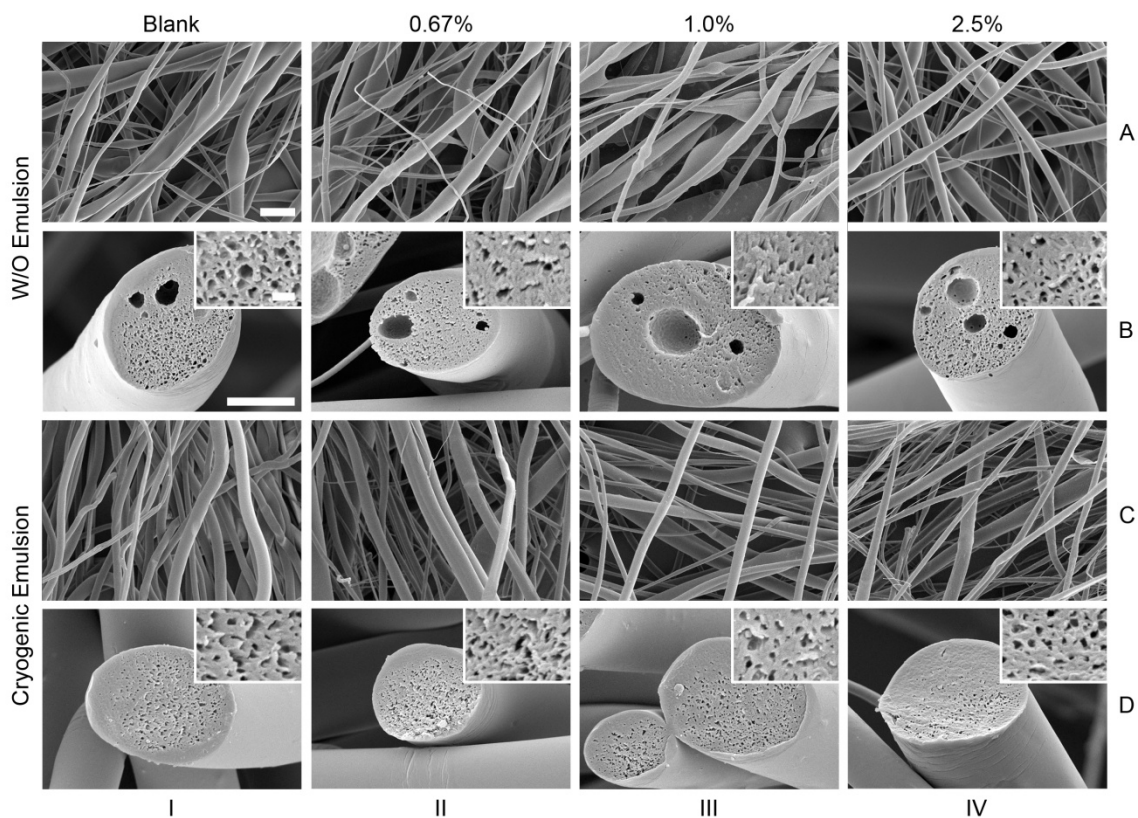


Figure 2.6: Scanning electron micrographs of blank and heparin-loaded PLLA microfibers prepared from W/O emulsion and cryogenic emulsion. The fabrication parameters used were a 10% (w/v) spin dope concentration, a 375 mL nonsolvent bath of 2:3 PE to SO ratio, and a stirring rate of 500 rpm. Group identifiers are shown in the top and left margins. Scale bars, (Row A, C) 50 μm (300x magnification), (Row B, D) 10 μm (300x magnification) and (insets) 1 μm (10kx magnification).

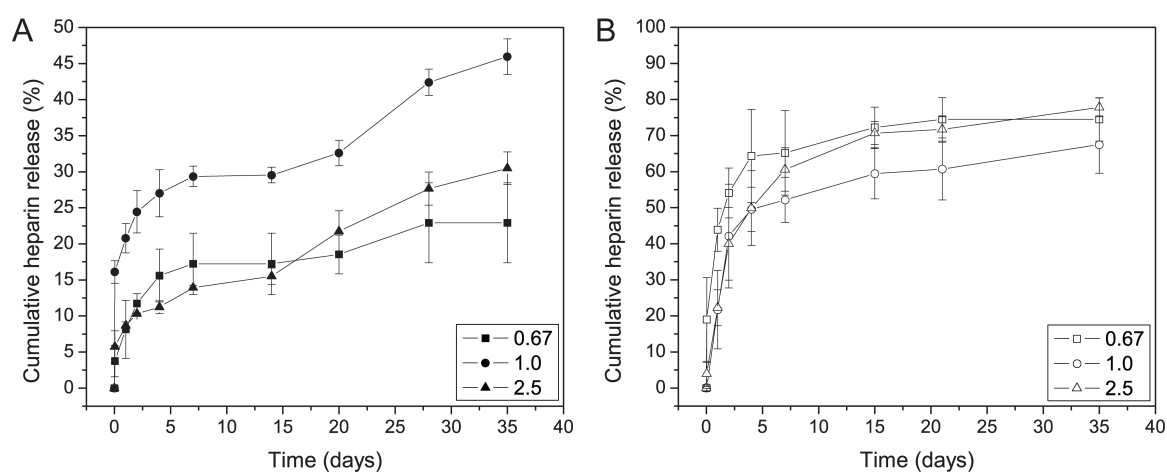


Figure 2.7: Heparin release profiles of PLLA microfiber scaffolds prepared from (A) W/O emulsion and (B) cryogenic emulsion spin dope solutions. Release is expressed as percentage of actual loading (error bars are mean $\pm$ SD, $n = 3$).

Chapter 3

Multifunctional polymeric microfibers with prolonged drug delivery and structural support capabilities

The strength and stability of hybrid fiber delivery systems, ones that perform a mechanical function and simultaneously deliver drug, are critical in the design of surgically implantable constructs. We report the fabrication of drug-eluting microfibers where drug loading and processing conditions alone increase microfiber strength and stability partially due to solvent-induced crystallization. Poly(L-lactic acid) microfibers of 64 ± 7 μm diameters were wet spun by phase inversion. Encapsulation of a model hydrophobic anti-inflammatory drug, dexamethasone, at high loading provided stability to microfibers, which maintained linear cumulative release kinetics up to 8 weeks in vitro. In our wet spinning process, all microfibers had increased crystallinity (13–17%) in comparison to unprocessed polymer without any mechanical stretching. Moreover, microfibers with the highest drug loading retained 97% of initial tensile strength and were statistically stronger than all other microfiber formulations, including control fibers without drug. Results indicate that the encapsulation of small hydrophobic molecules

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(<400 Da) may increase the mechanical integrity of microfilaments whose crystallinity is also increased as a result of the process. Multifunctional drug-eluting microfibers can provide an exciting new opportunity to design novel biomaterials with mechanical stability and controlled release of a variety of therapeutics with micron-scale accuracy.

3.1 Introduction

Polymeric fibers have many medical uses, such as surgical sutures, dialysis devices, wound dressings and tissue engineering scaffolds. Advances in polymer and drug delivery sciences have led to the evolution of engineered fibers for use as drug delivery vehicles. Over the past decade, research towards the design of therapeutically active fibers has increased [1-6]. While there are many techniques to make polymeric fibers, only a small subset of these methods is suitable for drug encapsulation. Wet spinning is one such technique that lends itself to drug delivery technologies since it can be done at ambient temperatures and is most similar to conventional microsphere-based encapsulation techniques. Wet spinning also produces micron-sized fibers that have the potential to be woven, knitted, braided, or embroidered into macro-level scaffold superstructures for the clinical reconstruction of damaged tissues / organs.

Wet spun microfiber delivery systems have been achieved by impregnating therapeutics into the core of hollow microfibers, entrapping therapeutics within microfibers, and chemically crosslinking or adsorbing therapeutics to the surfaces of microfibers. A broad range of biologically active therapeutics including antibiotics [7-9], heparin [10], proteins [6, 11-14], growth factors [4, 15], genes [16, 17] , and even viruses [18, 19] have been incorporated into wet spun microfibers. Recent efforts have focused on the biocompatibility and the biological activity of therapeutics delivered from wet spun microfibers. However, the effect of drug incorporation on the mechanical integrity of wet spun microfibers is not well understood [9, 20-22]. The mechanical properties of engineered microfibers for drug delivery and tissue engineering applications are

important to their functionality *in vivo*. For clinical applications, therapeutic fibrous drug delivery implants must retain structural integrity during surgical implantation, and provide mechanical and pharmaceutical support *in vivo* throughout the process of tissue integration and implant degradation which can occur over several months up to a year. Mechanical cues are also important for the organization, growth, and maturation of reconstructed tissues. Even small changes in the mechanical properties of the local microenvironment have been shown to influence cell behaviors by altering cell-cell and cell-matrix interactions [23].

In this study, we examined the dynamic mechanical properties of drug-eluting wet spun microfibers. Dexamethasone (DXM), a potent anti-inflammatory drug served as the model therapeutic for this study. The local delivery of DXM has been shown to reduce adverse cellular immune responses to a variety of implanted biomaterials [24-28]. Targeted DXM delivery may be beneficial for reducing unwanted inflammatory responses to fibrous implants and improving tissue integration. Here we describe the drug-polymer interactions and the effects of DXM encapsulation and release on the physical and thermal properties of wet spun PLLA microfibers up to eight-week incubation.

3.2 Materials and methods

3.2.1 Materials

Poly(L-lactic acid) (PLLA, $M_w \sim 120,000$) was purchased from Lactel Biodegradable Polymers (Birmingham, AL). Dexamethasone (DXM) and HPLC grade acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO). Analytical grade petroleum ether (PE), dichloromethane (DCM), tetrahydrofuran (THF), and glacial acetic acid were purchased from Fisher Scientific (Pittsburgh, PA).

3.2.2 Fabrication of dexamethasone-loaded microfibers

PLLA (7.5% w/v) DXM-loaded (1.0%, 2.4%, 4.8% w/w) and unloaded (0% w/w) microfibers were wet spun by phase inversion. PLLA (526.9 ± 0.3 mg) was dissolved in a co-solvent ratio of 6:1 (v/v) DCM to THF. DXM was added to the co-solvent solution at varying concentrations up to 3.6 mg/mL, near its maximum solubility. The addition of THF was necessary to increase DXM solubility within the spin dope. The polymer / drug solution was loaded into a 5 mL syringe fitted with a 22-gauge spinneret and placed in a syringe pump with a solution flow rate of 0.06 mL/min. Since DCM and THF are miscible with petroleum ether, the immersion of the spinneret into the coagulation bath resulted in continuous microfiber formation and subsequent encapsulation of DXM. A rotating mandrel placed above the spin bath was used to collect microfibers for further experimentation. Each formulation in this study was characterized from multiple regions of meter-long microfiber bundles spun at the same time from one spin dope solution.

3.2.3 Microfiber morphology

Scanning electron microscopy (SEM) was used to study the surface and cross-sectional morphology of wet spun microfibers. Fiber samples were placed on double-sided carbon tape and coated with a 50-100 Å layer of gold-palladium using a sputter coater (Emitech, Kent, England). SEM was conducted using a Hitachi S-2700 (Tokyo, Japan) microscope with an accelerating voltage of 8 kV and a working distance of 12 mm. Micrographs were collected using a Quartz PCI digital imaging system. The average microfiber diameter was measured using NIH ImageJ software (Bethesda, MD) from 10 fields of view taken at 100x magnification. The porosity was also measured from cross-sectioned microfibers and calculated as the pore area divided by the total cross-sectional area.

3.2.4 Determination of microfiber drug loading

Drug-loaded and control fiber bundles (5.0 ± 0.3 mg) were dissolved in 5 mL of 6:1 (v/v) DCM to THF until a clear solution was obtained. The encapsulation efficiency of DXM-loaded microfibers was determined by UV absorbance at 239 nm using quartz cuvettes to minimize background noise at the reading frequency. The percentage of drug encapsulated was calculated as the amount of DXM detected in microfibers relative to the total amount of drug added to the spin dope solution. Two samples from each formulation were assayed in duplicate.

3.2.5 Dexamethasone release study

As-spun microfibers from each batch (21.0 ± 2.0 mg) were incubated with 2 mL phosphate buffered saline (PBS, pH 7.4) in capped microcentrifuge tubes and kept at 37 °C. At each time point, 1 mL of the releasate was removed and replaced with fresh PBS. Prior to release analysis, DXM releasates were lyophilized and reconstituted in a mobile phase of 52:48 (v/v) 2 mM acetate buffer (pH 4.8) to acetonitrile. DXM detection was performed using a 3.9 x 150 mm Novapack C-18 column with a mobile phase flow rate of 1 mL/min at 240 nm after an average elution time of 2 minutes. Drug concentration was determined by comparing the area under the peak at the expected elution time with a calibration curve constructed from samples of known concentration.

3.2.6 Degradation study

PLLA microfibers (23.0 ± 2.0 mg) prepared by wet spinning were weighed in microcentrifuge tubes and incubated in 2 mL PBS (pH 7.4). The tubes were capped and placed at 37 °C. The duration of the degradation study was eight weeks with weekly terminal time points. At each sampling interval, the PBS solution was removed and the

supernatant pH measured using a Corning pH meter (Medfield, MA). The remaining microfiber bundles were then washed three times in distilled water and lyophilized for 24-48 hours.

3.2.7 Differential scanning calorimetry (DSC) and hyper DSC analysis

The thermal properties of microfibers from terminal time points were analyzed for thermal transitions using a DSC-7 (Perkin Elmer) equipped with an Intracooler 2 intercooling system (Perkin Elmer). Samples were subjected to: cooling to -25°C; heating to 250°C at 10°C/min; cooling to -25°C at 10°C/min; and reheating the sample to the upper limit again at the initial rate. Glass transition temperature, melting temperature, and change in enthalpy of the melt were measured from the resulting thermograms. The percent crystallinity (X_c) was also calculated using equation 3.1:

$$X_c = \frac{\Delta H_m}{\Delta H_{PLLA}} \times 100 \quad (\text{equation 3.1})$$

where ΔH_m is the enthalpy of melting of the samples and ΔH_{PLLA} (93.7 J/g) is specific heat of melting of a 100% crystalline PLLA as reported in the literature [29]. The dispersion of solid drug particles not solubilized within the polymer matrix was also analyzed using a DSC-8500 (Perkin Elmer) capable of hyper DSC. Samples were subjected to heating from 20°C to 310°C at 200°C/min and compared to the thermogram of free DXM from the manufacturer.

3.2.8 X-ray diffraction (XRD) analysis

The structural properties of PLLA microfiber formulations were determined using an automated X-ray diffractometer (Siemens Diffraktometer D5000) with a Cu K α ($\lambda = 1.54\text{\AA}$) radiation. The diffraction angles (2θ) ranged from 6° to 60° with sampling

intervals of $0.02^\circ \text{ s}^{-1}$. Diffraction signal intensity was monitored and processed using DiffracPlus Software (Bruker AXS).

3.2.9 Mechanical properties of PLLA microfibers

Uniaxial tensile testing was used to characterize the mechanical properties of wet spun PLLA microfibers. Tests were performed at ambient temperature, humidity, and pressure using an Instron materials testing machine (Model 4442). Gauge length and elongation rate were defined in accordance to United States Pharmacopeia standards for absorbable sutures [30]. Microfibers were secured onto paper frames (25 mm x 25 mm) with precut windows to define gauge length and region of loading. After clamping samples into the crossheads of the machine, the edges of each frame were cut leaving microfibers intact. Samples were loaded to failure at a constant elongation rate of 50 mm/min. Load cell measurements and displacement data from crosshead extension were converted into stress-strain data. Ultimate tensile strength, strain at failure, and elastic modulus, were calculated from the resulting stress-strain curves.

3.2.10 Statistical analysis

One-way analysis of variance (ANOVA) tests were performed on mechanical data for wet spun microfibers using SPSS v.19 statistical software (Chicago, IL). *Post hoc* analyses were carried out using the Tukey multiple comparisons test. A p value of less than 0.05 was considered to be significant.

3.2. Results and discussion

3.3.1 Microfiber extrusion and morphology

Our interest is in developing micron-scale fibers for surgical reconstruction. Local, tunable drug diffusion could be used to create anti-neoplastic or immune-privileged boundaries; in the context of reconstructive surgeries it would be highly advantageous to control local wound healing, fibrosis, scarring, and injury responses, including calcification. In vascular reconstructions, modulation of anastomotic healing could improve patency rates for microvascular repairs and their presence in blood vessels opens the possibility for prolonged, regional, systemic delivery of therapeutic molecules via the circulation for targeted downstream effects.

This study aims to gain an understanding of how drug encapsulation and processing conditions affect the mechanical integrity of microfibers. The overarching goal of this research is to create microfibers that can perform a surgically required mechanical function and simultaneously deliver drugs. To evaluate the drug-drug and drug-polymer interactions, 1.0, 2.4, and 4.8% (w/w) DXM were wet spun into PLLA microfibers. In the spinning of semi-crystalline polymers, it is well known that the crystalline regions of the polymer contribute to strength. Thus, PLLA was selected for wet spinning because of its material properties.

PLLA spin dope solutions extruded into the petroleum ether coagulation bath underwent phase inversion within centimeters of the spinneret, forming an opaque white microfilament. Residence time was 5-10 seconds before microfibers were collected around a rotating mandrel placed above the spin bath. Samples were strong and could be spun easily, indicating quite fast and effective curing within this short time span. Representative SEMs of microfibers are shown in **Figure 3.1 (Row A, B)**. Wet spinning conditions produced 64.3 ± 7.0 μm diameter microfibers with skin-core structures. Skin-core structure is common to wet spinning, and is caused by rapid

surface coagulation leading to the entrapment of solvent and nonsolvent within the precipitating microfilament [31]. Porous structure is formed once solvent and nonsolvent are evaporated after microfilament solidification. The solvents involved in the formation of the fiber play a major roll in further crystallizing the polymer by solvent-induced crystallization (SINC) (Section 3.5). DXM-loaded and control microfibers had round cross-sectional geometry and relatively porous morphology with similar interconnectivity. The average percent porosity of microfibers was 17.0 ± 1.3 with an average pore size area of $1.26 \pm 0.06 \mu\text{m}^2$ as determined from the micrographs of cross-sectioned microfibers. Microfibers displayed micron-rough surface topography with spherical protrusions similar to studies by Nelson et al. [32]. Spherical structures could be spherulites (**Figure 3.1**) forming as the polymer is cured [33-35]. Maltese cross patterns were also observed under cross-polarized light (**Figure 3.2**).

Incubation time had no significant effect on microfiber shape and cross-sectional morphology (**Figure 3.1, Row C, D**). Diameters did not change more than $5.0 \mu\text{m}$ after incubation in PBS for eight weeks. The average microfiber diameters of 0% (control), and 1.0%, 2.4%, and 4.8% (w/w) DXM-loaded samples throughout the degradation study were $62.8 \pm 6.8 \mu\text{m}$, $65.7 \pm 5.8 \mu\text{m}$, $65.6 \pm 5.8 \mu\text{m}$, and $62.9 \pm 8.3 \mu\text{m}$, respectively. While an occasionally large pore was observed among all sample cross sections, the overall porosity of fibers did not change with time. At eight-week incubation, microfibers had an average percent porosity of 18.3 ± 3.1 and an average pore size area of $1.24 \pm 0.09 \mu\text{m}^2$. This data implies PLLA microfiber degradation has barely commenced over the incubation period. Our findings are consistent with the slow degradation of wet spun PLLA fibers as reported by others [36, 37].

3.3.2 Dexamethasone encapsulation and release kinetics

To determine the encapsulation efficiency and drug distribution within wet spun microfibers, two separate regions of microfiber bundles collected from the coagulation bath were assayed. 1.0-4.8% (w/w) DXM was encapsulated with relatively high efficiency (76-93%) as shown in **Table 3.1**. DXM and PLLA were dissolved in 6:1 (v/v) DCM to THF before precipitation in the petroleum ether bath. The coagulation rate of fibers was nearly instantaneous, resulting in little overall drug loss during polymer solidification. 4.8% (w/w) DXM-loaded microfibers had slightly decreased encapsulation efficiency in comparison to 1.0% and 2.4% (w/w) formulations. The observed decrease with 4.8% (w/w) drug loading could be because we have reached the limit for drug loading of 7.5% (w/v) PLLA microfibers; drug loadings greater than 4.8% (w/w) resulted in significant drug loss as shown with 10% (w/w) theoretical DXM loading in **Table 3.1**. For these reasons, only formulations with 1.0%, 2.4% and 4.8% (w/w) theoretical loading were characterized in this study. No significant variations in drug loading were observed between different sampling regions of meter-long microfibers bundles, signifying a homogeneous distribution of drug.

Release profiles of DXM-loaded fibers are shown in **Figure 3.3A**. Each fiber formulation exhibited little burst, followed by linear cumulative percent DXM release starting at day 3 up to eight-week incubation ($R^2 = 0.92-1.0$). Formulations with 0.9% and 2.2% (w/w) actual DXM loading released less than 1% and 2% of total drug encapsulated during the first three days. In contrast, 3.6% (w/w) DXM-loaded fibers released 5% of total drug encapsulated during the first three days and maintained a faster rate of drug release throughout the course of the study. Greater initial release of DXM from 3.6% (w/w) actual drug-loaded fibers could be due to drug located near the surface of microfibers. Increased drug loading led to an overall increase in drug release, with lower loadings displaying similar release rates over time. This suggests that at lower loadings, the drug is well encapsulated within the polymer matrix with little drug

near the surfaces of microfibers. Drug loss during encapsulation at high loadings could also have attributed to drug distribution more towards the surfaces of microfibers. The cumulative percent DXM released after eight weeks was 5.54 ± 0.12 for 0.9% (w/w) actual drug loading, 4.62 ± 1.08 for 2.2% (w/w) actual drug loading, and 27.82 ± 5.13 for 3.6% (w/w) actual drug loading. The absence of morphological changes observed by SEM implies drug release is not predominately controlled by polymer degradation, but rather by dissolution and diffusion of drug into the supernatant. At low loadings, drug release is likely due to the dissolution of the drug, whereas at higher loadings, drug release is a combination of dissolution and diffusion. Since the 3.6% (w/w) actual drug-loaded microfibers released 202.5 μg after eight weeks, nearly ten times the amount of drug relative to the 2.2% (w/w) formulation that released 20.6 μg , a significant portion of drug release must be due to diffusion. Mack et al. have also studied release of DXM from microfibers [13, 20]. At eight weeks, wet spun PLGA (50:50) microfibers released 60-90% total encapsulated drug primarily due to polymer degradation. According to the manufacturer, the degradation rate of the raw PLLA used in this study is >24 months. Therefore, we believe the release behavior from our microfiber delivery system over the course of eight-week incubation is predominantly due to the hydrophobic nature of DXM.

The controlled, sustained release of dexamethasone may constitute a clinically important therapeutic modality for biomaterial-driven inflammation. Previous studies have shown that the local, targeted delivery of dexamethasone over extended periods of time (months) reduces immune responses to implanted biomedical devices and polymeric biomaterials [24, 25, 27, 38]. The effective therapeutic ranges of drug evaluated in previous studies ranged from 0.17 to 30 $\mu\text{g}/\text{day}$, depending on the size and material of the implant. The average daily release rates after initial burst for 20 mg microfiber bundles from this study were around 1-2 $\mu\text{g}/\text{day}$ for 0.9 and 2.2% actual DXM loading and 15 $\mu\text{g}/\text{day}$ for 3.6% actual DXM loading. Therefore, it would be possible to tune DXM release rates within the therapeutic ranges of drug treatment by combining

multiple formulations into multifilament yarns and by simply altering scaffold architecture [39]. For example, weft-knitted scaffolds require more yarn material in the assembly of macrometric structures and thus are capable of delivering a greater dose of therapeutics than warp-knitted scaffolds of similar dimensions.

3.3.3 Supernatant pH characteristics

The diffusion of PLLA oligomer was analyzed by measuring the supernatant pH over degradation time. The degradation of PLLA has been well established [40]. As PLLA degrades, it yields L(+)-lactic acid, a naturally occurring stereoisomer of lactic acid. The lactic acid monomers from the hydrolytic de-esterification of PLLA enter the carboxylic acid cycle and are excreted as water and carbon dioxide. For this reason PLLA is widely used in drug encapsulation. The supernatant pH of terminal time points was determined to compare the relative changes of DXM-loaded microfibers in comparison to control microfibers with no drug. All formulations displayed similar decrease in pH (**Figure 3.3B**). At eight-week incubation, 0% (control), 1.0%, 2.4% and 4.8% (w/w) formulations decreased in pH by 0.95, 1.25, 1.56, and 1.37, respectively. The inclusion of DXM could potentially increase the hydrophobicity of microfibers, resulting in little pH changes over time. It is important to note that the PBS was not changed and each time point was terminal for this study only. Crow et al. studied supernatant pH changes as a function of bovine serum albumin (BSA) release from wet spun PLLA microfibers [36]. At 15-week incubation, control microfibers decreased in pH by 1.70, with buffer changes occurring at each time point. Given these findings, it is unlikely that DXM release from microfiber samples characterized in release studies was mediated by PLLA degradation.

3.3.4 Thermal analysis

The morphology of blank and degraded microfibers was studied using DSC. Representative DSC traces of 4.8% (w/w) DXM-loaded microfibers after fabrication and throughout degradation are presented in **Figure 3.4A**. Thermal transitions calculated from the first heating scan are listed in **Table 3.2**. The first interesting observation we have found was that relative PLLA crystallinity, as a result of wet spinning, was substantially increased. Extrusion of PLLA alone increased relative crystallinity by 17% in comparison to raw polymer from the manufacturer. Drug-loaded microfibers also had an increase in relative crystallinity by 13 -16% after wet spinning. Several factors could be contributing to this effect, including the solvents, nonsolvent, and residence time during fabrication, which could lead to solvent-induced crystallization (SINC) (**Figure 3.5A**) [41]. It is well known that many polymers undergo SINC. When a polymer (semi-crystalline) is exposed to a solvent between the T_g and the T_m , crystallization becomes kinetically favorable. We believe the choice of the solvents and the solubility parameters of solvent ($\delta_{\text{DCM}} = 20.2 \text{ MPa}^{1/2}$, $\delta_{\text{THF}} = 18.6 \text{ MPa}^{1/2}$) and nonsolvent ($\delta_{\text{PE}} = 14.8 \text{ MPa}^{1/2}$) in our system affect the rate of solvent removal, solidification, and eventually the degree of crystallinity of PLLA ($\delta_{\text{PLLA}} = 20.7 \text{ MPa}^{1/2}$) microfilaments. In wet spinning, an initially homogeneous polymer solution is extruded into a coagulation bath that induces phase inversion by counter-diffusion of solvent and nonsolvent. Phase separation of the polymer solution is then initiated and the liquid spin dope stream begins to precipitate into a solid microfilament. In general, it is possible to associate the counter-diffusion of solvent and nonsolvent with the thermodynamic stability of extruded spin dope solutions [34]; the precipitation of polymeric solutions is typically faster with a higher diffusion of solvent in nonsolvent. Rissanen et al. wet spun microfibers with similar residence time by phase inverting PLDLA ($\delta_{\text{PLDLA}} = 23.3 \text{ MPa}^{1/2}$) dissolved in DCM into an ethanol (EtOH) spin bath ($\delta_{\text{EtOH}} = 26.6 \text{ MPa}^{1/2}$) resulting in a 29% decrease in crystallinity [42]. The difference in the solubility parameter between the solvent / nonsolvent, in the last example, is about 6 units,

which makes EtOH a better nonsolvent for the microfiber than petroleum ether. Thus, faster precipitation may not have allowed for SINC conditions to occur. The effects of drug loading on the SINC in our system will be further discussed in Section 3.5.

No significant differences in glass transition temperature after fabrication could be observed between the 1.0% and 2.4% (w/w) DXM-loaded samples in comparison to control microfibers (**Table 3.2**). However, 4.8% (w/w) DXM-loaded samples showed a slight decrease in glass transition temperature. This, at first glance, suggests that DXM does not act as plasticizer and interacts with the polymer matrix, or that there is too little drug to detect changes in glass transition temperatures. Since there appeared to be little DXM interaction with the amorphous regions of the polymer, as judged by the minor changes in glass transition, hyper DSC was used to study the dispersion of solid drug within wet spun microfibers. Conventional DSC techniques limit the sensitivity to measure the drug melting temperature [43]. Slow heating scan rates of 10°C/min allot sufficient time for molecules within the crystal lattice to respond to thermal transitions and further solubilize within the polymer matrix. Hyper DSC uses scanning rates of >100°C/min to inhibit further solubilization of drug within the matrix. Therefore, the fraction of drug solubilized within the matrix does not contribute to the melting endotherm associated with the dispersed drug fraction. Hyper DSC thermograms revealed no melting point at 300°C for DXM (**Figure 3.4B**), indicating the drug was either amorphous or formed a solid solution within PLLA microfibers and precipitated in an amorphous state.

The polymer crystallinity and glass transition temperatures of degraded samples taken from terminal time points were also evaluated using DSC. If major degradation did occur mostly the amorphous regions would disappear, leaving fragmented samples with very high crystallinity. Thus we would expect to see an increase in crystallinity at the early stages of incubation. However, DXM-loaded samples had minimal changes in crystallinity over time, signifying minor polymer degradation. Interestingly, the glass transition temperature of all microfibers slightly increased with incubation. It is well

known that when polymers are annealed the glass transition often increases, which is related to a reduction in free volume and possibly segmental rearrangement. The glass transition temperature for the second heating scan of the raw polymer is 47.6°C. Since the glass transition temperature of the second heating scan is close to the incubation temperature (37°C), the mobility of the polymer chains could have increased with incubation time. An increase in chain mobility could result in the formation of crystalline structures similar to studies by Zong et al. [44]. Constrained amorphous chains from thermally induced crystallization could have resulted in the increase in glass transition temperature with incubation. Secondary DXM-polymer interactions already existing from fabrication could have been further increased as some of the polymer degrades. Even minor PLLA degradation during incubation would result in free carboxylic and hydroxylic groups that are capable of forming secondary interactions with the drug (**Figure 3.5B**). This effect could have also contributed to amorphous chain restructuring and a reduction in free volume. Additionally, the specific interactions between PLLA and DXM could be a factor in the slow release of drug from our delivery system, as was also suggested in a study of DXM release from PLLA films [45].

3.3.5 XRD analysis

XRD was used to further investigate the physical state of the drug in wet spun microfibers. No crystalline DXM was detected in any of the microfiber formulations (**Figure 3.6**). The absence of the specific diffraction of DXM in the x-ray diffractogram of drug-loaded PLLA microfibers indicates that DXM is present as either an amorphous drug or as a solid solution within the amorphous regions of the polymer (molecular dispersion). Gómez-Gaete et al. found similar DXM incorporation into PLGA nanoparticles prepared by solvent evaporation [46]. Evaluation of the four formulations from our study reveals control microfibers without drug have a less ordered state than microfibers loaded with drug. As drug loading is increased the crystalline order increases

and a new crystalline peak appears in the diffraction pattern as shown with 1.0, 2.4, and 4.8% drug loading in **Figure 3.6**. Since microfibers had short residence time (5-10 seconds) in the nonsolvent, residual solvent is likely still within the precipitating filament after it has been collected from the spin bath. SINC may explain the increase in the ΔH_m of the blank fibers as compared to pure polymer. However, the change in the PLLA XRD morphology with the drug-loaded samples may be a result of the solvent-drug interaction during SINC that keeps the glass transition lower for a longer time thus allowing for further crystallization to take place after the precipitation has started. We believe that this is the origin of the new peaks in **Figure 3.6** for the drug-loaded formulations. Incidentally, the same control fibers left in the nonsolvent bath for a longer residence time revealed the same third peak (**Figure 3.7**). We think this could be the reason for the higher degree of crystallinity that we observe in all our samples.

3.3.6 Mechanical properties

To characterize the mechanical properties of wet spun microfibers, samples were loaded in uniaxial tension until failure. The results of mechanical analysis are shown in **Figure 3.8**. The mechanical properties of microfibers were consistent with that of wet spinning [21, 32]. Stress-strain curves for microfibers from each formulation displayed large plastic deformation until failure. Formulations also had similar elastic moduli (**Figure 3.8A**). Conversely, the ultimate tensile strength (UTS) and the total elongation before tensile failure (strain at failure) varied among drug-loaded samples relative to samples without drug. Microfibers with the highest drug loading, 4.8% (w/w) had the greatest tensile strength (15.6 ± 0.5 MPa) of drug-loaded formulations and were significantly stronger ($p < 0.01$) than control microfibers (**Figure 3.8B**). Drug encapsulation at low loadings, 1.0% and 2.4% (w/w), had similar tensile strengths in comparison to control microfibers. Strain at failure for formulations containing drug were significantly different, with increased drug encapsulation resulting in increased plastic deformation until

fracture (**Figure 3.8C**). The highest drug loading, 4.8% (w/w) was the only formulation with similar elongation until failure as control microfibers containing no drug.

Necking during the macroscopic plastic deformation of wet spun microfibers grows stronger due to the alignment of polymer chains with increasing stress. In semi-crystalline polymers, the amorphous regions of the polymer require less force to deform in comparison to the crystalline regions of the polymer. One explanation for increased strength and strain with the 4.8% (w/w) formulation is that dexamethasone is reinforcing the amorphous regions of the polymer. The process of wet spinning resulted in a marked increase in crystallinity as determined by DSC. If polymer chains are closely packed and parallel, crystallinity may increase mechanical strength when secondary bonding is enhanced. During incubation, dexamethasone molecules (392.8 Da) may form secondary interactions within the amorphous phase of the polymer, creating a reinforced composite material (**Figure 3.5C**). When small amounts of dexamethasone are encapsulated, the strain decreases until enough of the drug is encapsulated to exhibit similar strain at failure as control microfibers. Perhaps drug particles are acting as material defects at low encapsulation loading. Increasing DXM loading could increase drug-drug and drug-matrix interactions. Therefore, mechanical properties may be influenced by the secondary interactions, namely hydrogen bonds, between the polymer lattice and the drug particles.

To further elucidate the effect of drug loading and release on microfibers, mechanical properties were characterized throughout the degradation study. Tensile loss profiles of each formulation were generated from stress-strain profiles of terminal time points (**Figure 3.9A**). Microfibers with the highest drug loading, 4.8% (w/w), maintained 97.3% of initial tensile strength and were statistically stronger ($p < 0.05$) than all formulations after incubation for eight weeks (**Figure 3.9B**). Control, 1.0% and 2.4% (w/w) microfibers had a decrease in tensile strength in the first week, but then continued to maintain strength. At the end of eight weeks, the breaking strength

retention for control, 1.0%, and 2.4% (w/w) was 80.9%, 80.0%, and 81.3%, respectively. All formulations displayed a decrease in plastic deformation and strain at failure over time (**Figure 3.9C**), independent of drug content. This is consistent with the measurement of the specific heats reported in **Table 3.2**. The crystallinity of wet spun microfibers increases over time, causing microfiber embrittlement. Elastic moduli of samples and resistance to deformation under load were conserved over time (**Figure 3.9D**). Dexamethasone drug particles at 4.8% (w/w) may help maintain mechanical strength by secondary bonding adding to physical reinforcement. DXM may also preserve the structure of microfibers over time, which could be contributing to drug stabilizing the lattice and decreasing the permeation rate due to hydrophobic drug content.

Studies with melt spun PLLA microfibers encapsulating a small anti-inflammatory drug, curcumin (368.4 Da), have also shown preservation of mechanical properties with drug loading [47]. Curcumin impregnation at 10% (w/w) increased fiber tensile strength at failure for periods of incubation up to 36 days in comparison to control microfibers with no drug. Although melt spun microfibers produced higher tensile strengths than that of wet spun microfibers in this study, curcumin loaded microfibers only maintained 57% of their initial tensile strength at 25-day incubation. The processing conditions of wet spun microfibers fabricated by Mack et al. also possessed higher initial tensile strength than those used in our study [20]. However, after only 7-day incubation wet spun microfibers encapsulating levonixthan and dexamethasone lost nearly all of their tensile strength. Our data suggest dexamethasone incorporation increases mechanical integrity when microfiber crystallinity is also increased. Melt spinning is known to increase crystallinity due to the way polymer chains group themselves when forming a microfiber and therefore curcumin impregnation helped maintain mechanical properties. Conversely, wet spun microfibers fabricated using DMSO and water as solvent and nonsolvent reported no increase in crystallinity and therefore dexamethasone incorporation did not help maintain mechanical strength. Given these findings, the addition of small

hydrophobic drugs (<400 Da) may increase mechanical integrity of filaments whose crystallinity is increased as a result of fabrication. As the proximity of amorphous and crystalline regions increases, so does the possibility for strong secondary interactions between drug and polymer.

3.4 Conclusions

Wet spinning by phase inversion was used to fabricate mechanically stable dexamethasone-eluting PLLA microfibers. The prolonged release of drug at high loading, combined with the retention of mechanical strength demonstrates the effectiveness of our microfiber delivery system. While there are many methods for increasing the mechanical strength of wet spun microfibers by physical manipulation through post-drawing techniques, we report the fabrication of wet spun microfibers where processing conditions alone increase microfiber strength and stability partially due to SINC. Additionally, our delivery system is capable of achieving controlled, linear release of up to 28% total encapsulated drug after eight-week incubation. Dexamethasone incorporation into fibrous surgical implants could help to reduce innate immune response while providing the mechanical support necessary for tissue integration. Furthermore, the addition of small hydrophobic molecules, such as dexamethasone, into wet spun microfibers may serve as a means for maintaining the mechanical properties of microfilaments. A stable delivery system, one that can be physically manipulated and deliver controlled release of therapeutics while maintaining mechanical strength, would be beneficial for many applications in localized drug delivery and functional tissue engineering.

3.5 References

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3.6 Tables

Table 3.1: Encapsulation efficiency of microfibers prepared by wet spinning. Data are represented as mean $\pm$ SD.

DXM loading of dope solution (%w/w)	DXM loading of wet spun microfiber (%w/w)	DXM encapsulation efficiency (%)
1.0	0.9	89.0 ± 0.036
2.4	2.2	92.9 ± 0.084
4.8	3.6	75.8 ± 0.002
10.0	1.0	9.9 ± 0.010

Table 3.2: Summary of thermal properties and crystallinity from the first DSC heating scan of degraded microfibers.

Degradation time (weeks)	T_g (°C)	T_m (°C)	ΔH_m (J/g)	X_c (%)
<i>Control (0% DXM)</i>				
0	57.4	174.2	60.5	64.6
2	61.6	174.3	60.6	64.7
4	63.0	174.2	60.6	64.6
6	63.1	174.2	60.9	65.0
8	64.3	173.5	59.2	63.2
<i>1% DXM</i>				
0	59.5	173.7	59.2	63.2
2	55.9	173.4	59.2	63.1
4	63.3	174.7	56.0	59.8
6	63.8	174.0	61.1	65.2
8	62.2	173.9	61.4	65.5
<i>2.4% DXM</i>				
0	59.7	173.4	56.4	60.1
2	62.9	173.3	59.0	63.0
4	62.6	174.2	57.8	61.7
6	62.4	173.9	60.8	64.9
8	64.4	173.5	59.8	63.8
<i>4.8% DXM</i>				
0	54.3	173.7	57.0	60.8
2	59.4	174.0	57.5	61.4
4	62.2	174.0	58.9	62.8
6	63.2	173.4	58.4	62.4
8	63.6	174.0	57.9	61.8
<i>Unprocessed PLLA</i>				
n/a	52.6	174.7	44.5	47.4

T_g , glass transition temperature

T_m , melting temperature

ΔH_m , endothermic enthalpy of the melting peak

X_c , crystallinity degree

3.7 Figures

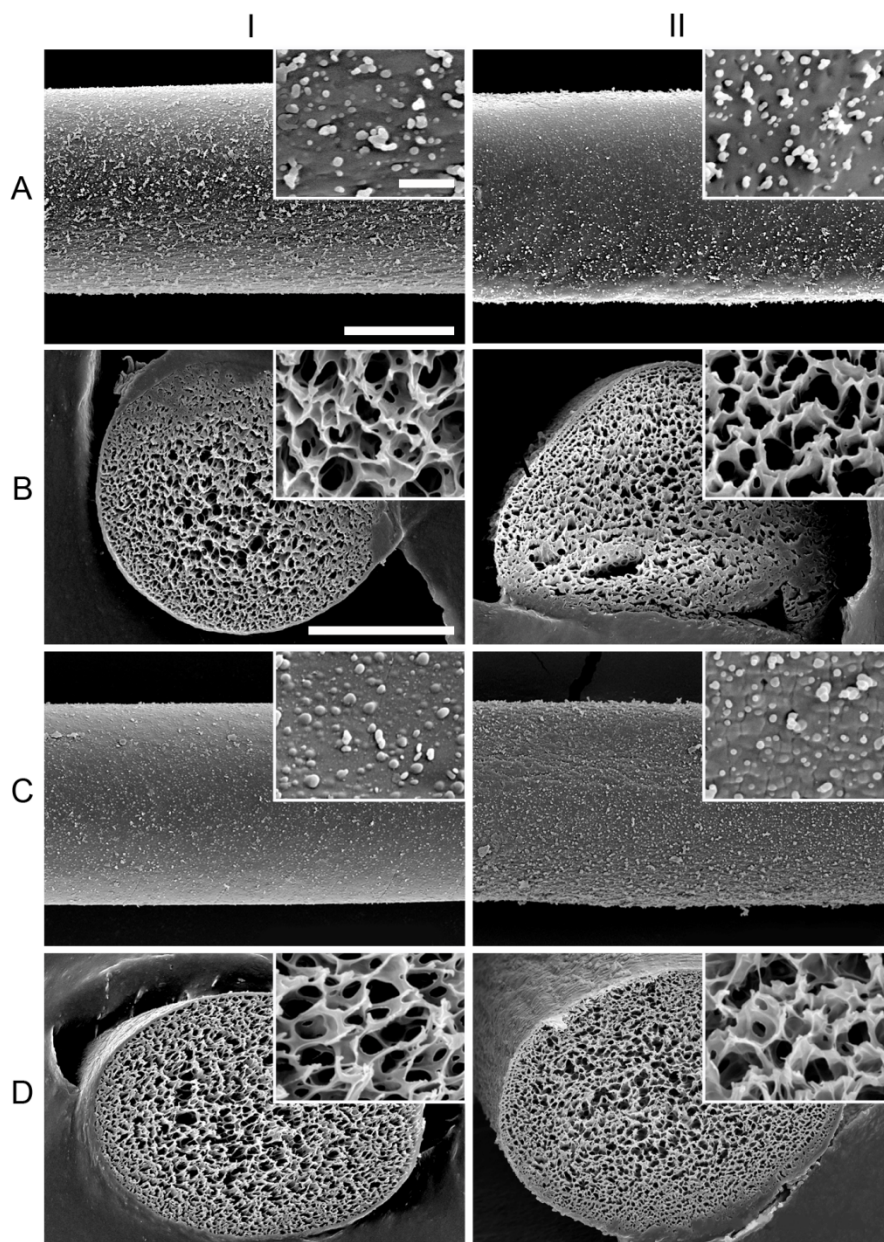


Figure 3.1: Surface and cross-sectional morphology of control and drug loaded microfibers after fabrication and eight-week incubation. Group identifiers are shown in the top and left margins. Scale bars, (Row A, C) 30 μm (1.0 $\times$ magnification); (Row B, D) 30 μm (1.3 $\times$ magnification); and (insets) 5 μm (5 $\times$ magnification).

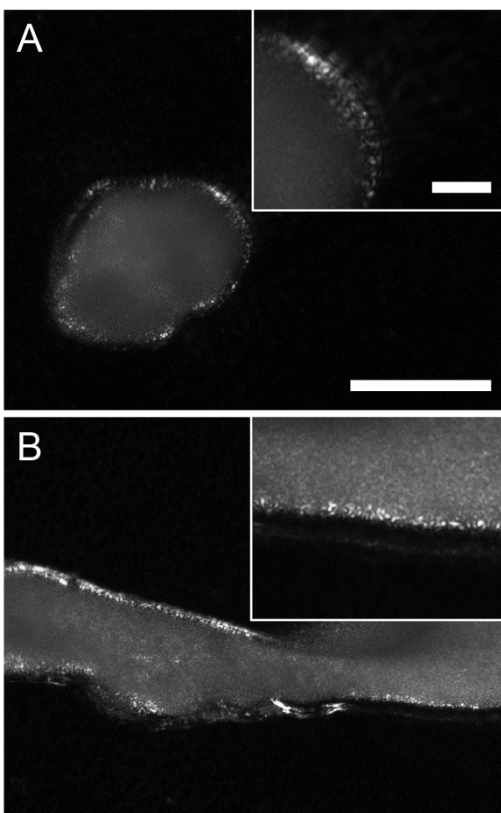


Figure 3.2: Polarized light micrographs depicting maltese cross patterns of 4.8% (w/w) DXM-loaded PLLA microfibers. (A) Axially and (B) orthogonally cross-sectioned microfibers. Scale bars, (A,B) 50 μm ; (insets) 10 μm .

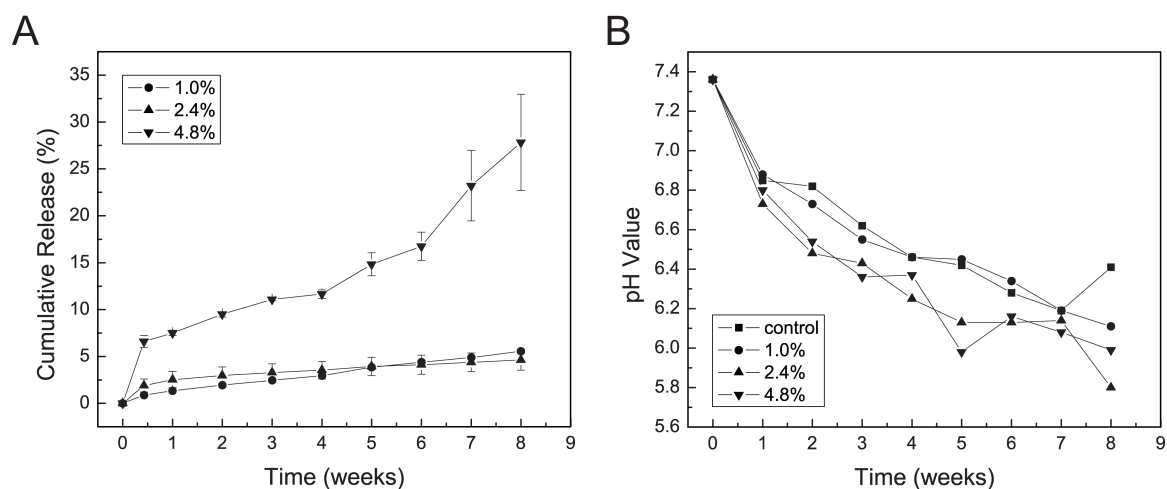


Figure 3.3: (A) Drug release profiles of wet spun PLLA microfibers loaded with 1.0% (●), 2.4% (▲), and 4.8% (▼) (w/w) DXM. Mean $\pm$ SEM are presented. (B) Plot of supernatant pH values versus degradation time for PLLA with incremental DXM loading in comparison to control microfibers without drug.

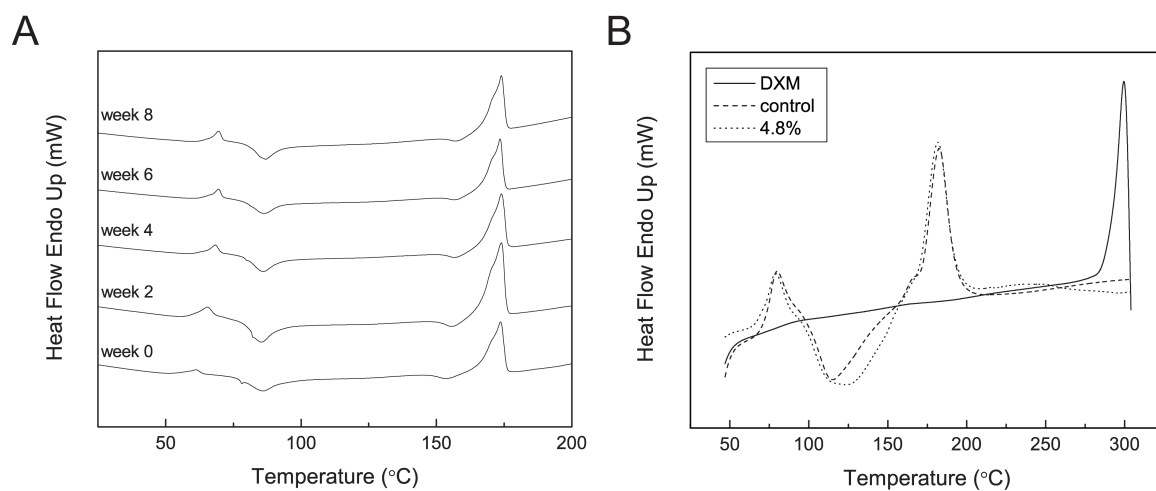


Figure 3.4: Thermal analysis of wet spun microfibers. (A) Representative DSC thermograms of degraded 4.8% (w/w) DXM-loaded microfibers. (B) Hyper DSC thermograms of control, 4.8% (w/w) DXM-loaded microfibers, and free drug. No melting endotherm is present at 300°C for drug-loaded microfibers.

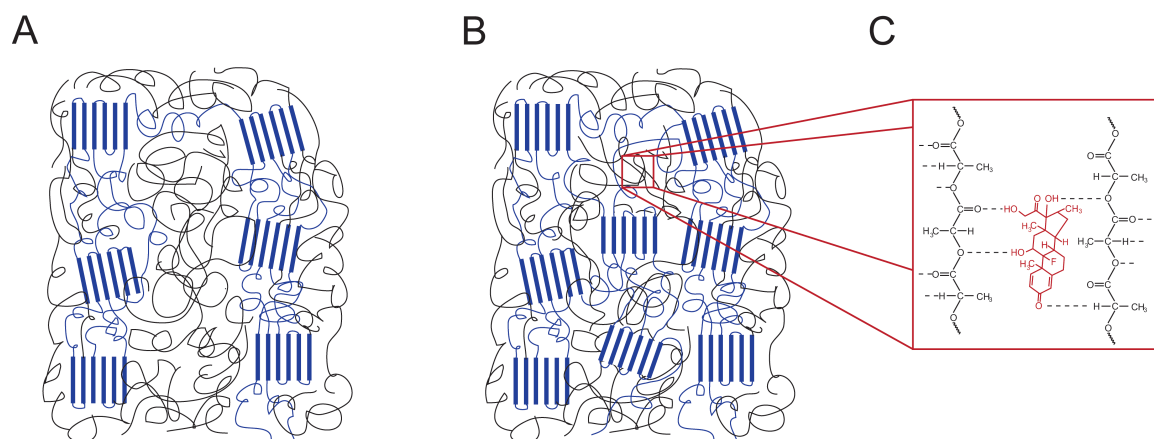


Figure 3.5: Schematic diagram of structure and morphology changes of wet spun PLLA microfibers. Scheme of (A) semi-crystalline polymer, (B) formation of new crystalline areas during solvent-induced crystallization (SINC), and (C) secondary interactions between DXM and PLLA. PLLA contains ester groups and DXM contains two carbonyl and three hydroxyl groups. Hydrogen bonding could occur between the carbonyl oxygen atoms in PLLA chains and the hydroxyl hydrogen atoms in DXM. Hydrogen bonding could also be enhanced during incubation as the amorphous regions of the polymer become more mobile.

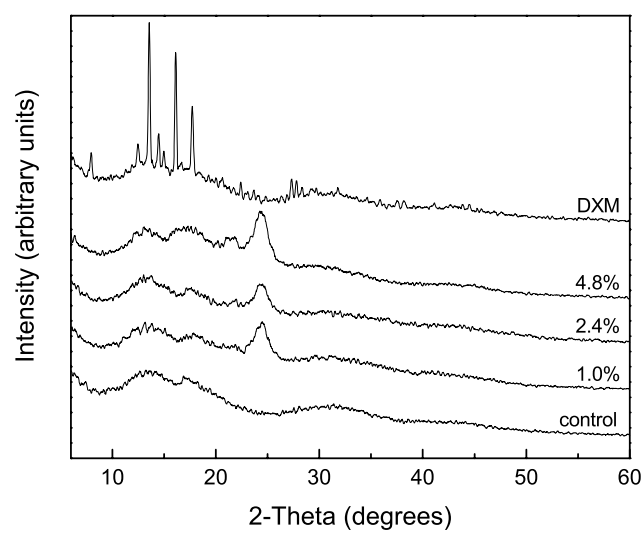


Figure 3.6: XRD patterns of control (0%), DXM-loaded microfibers, and free DXM.

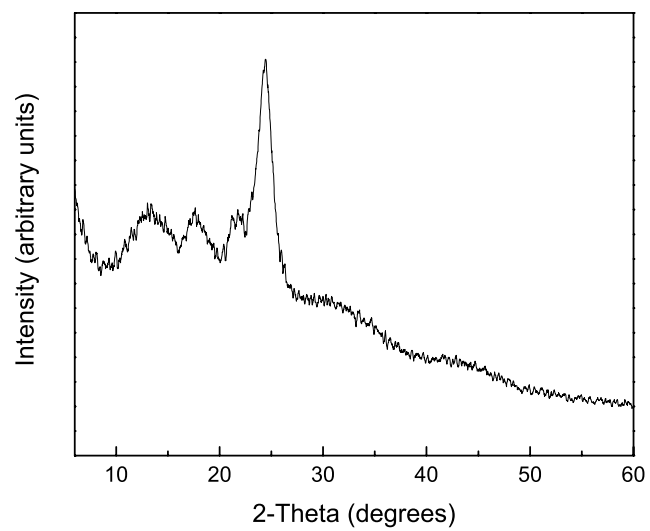


Figure 3.7: XRD pattern of control PLLA microfibers fabricated using a residence time of 1.5 hours.

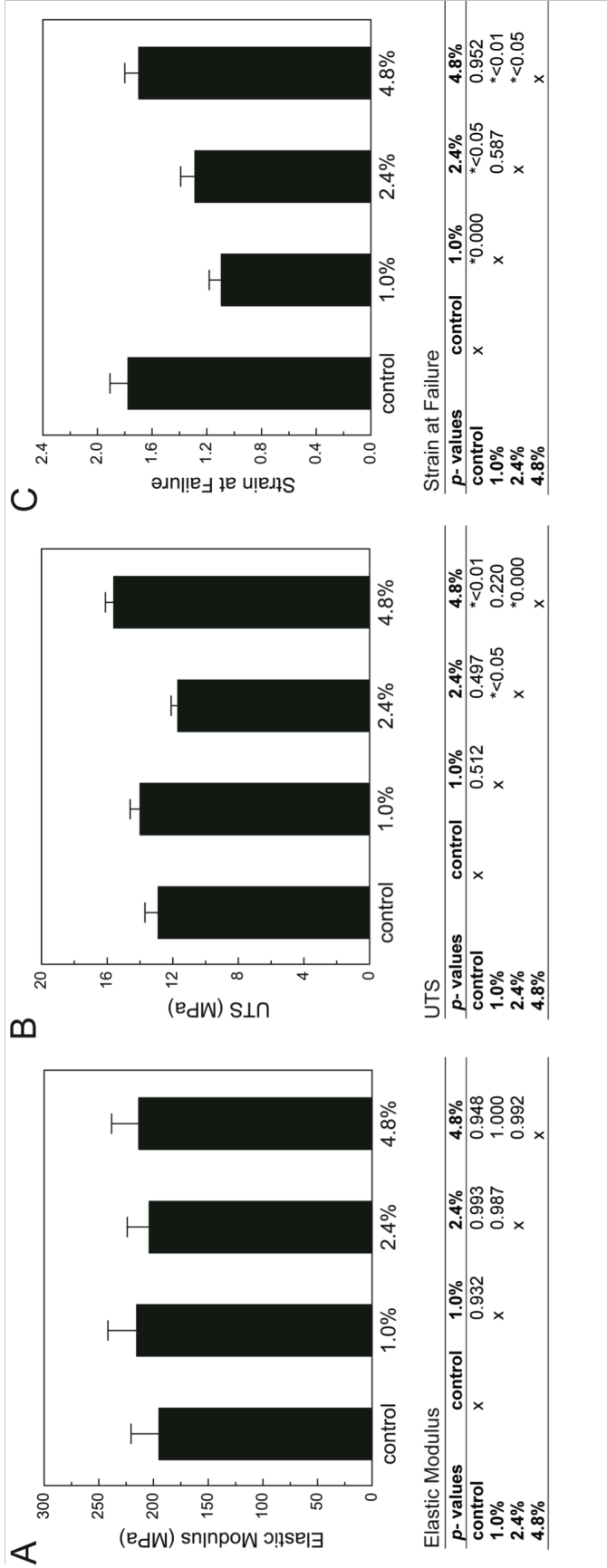


Figure 3.8: Mechanical properties of control (0%), 1.0%, 2.4%, and 4.8% (w/w) DXMM-loaded microfibers. (A) UTS, (B) elastic modulus, and (C) strain at failure were calculated for each formulation. Mean $\pm$ SEM are presented, * $p < 0.05$ by ANOVA.

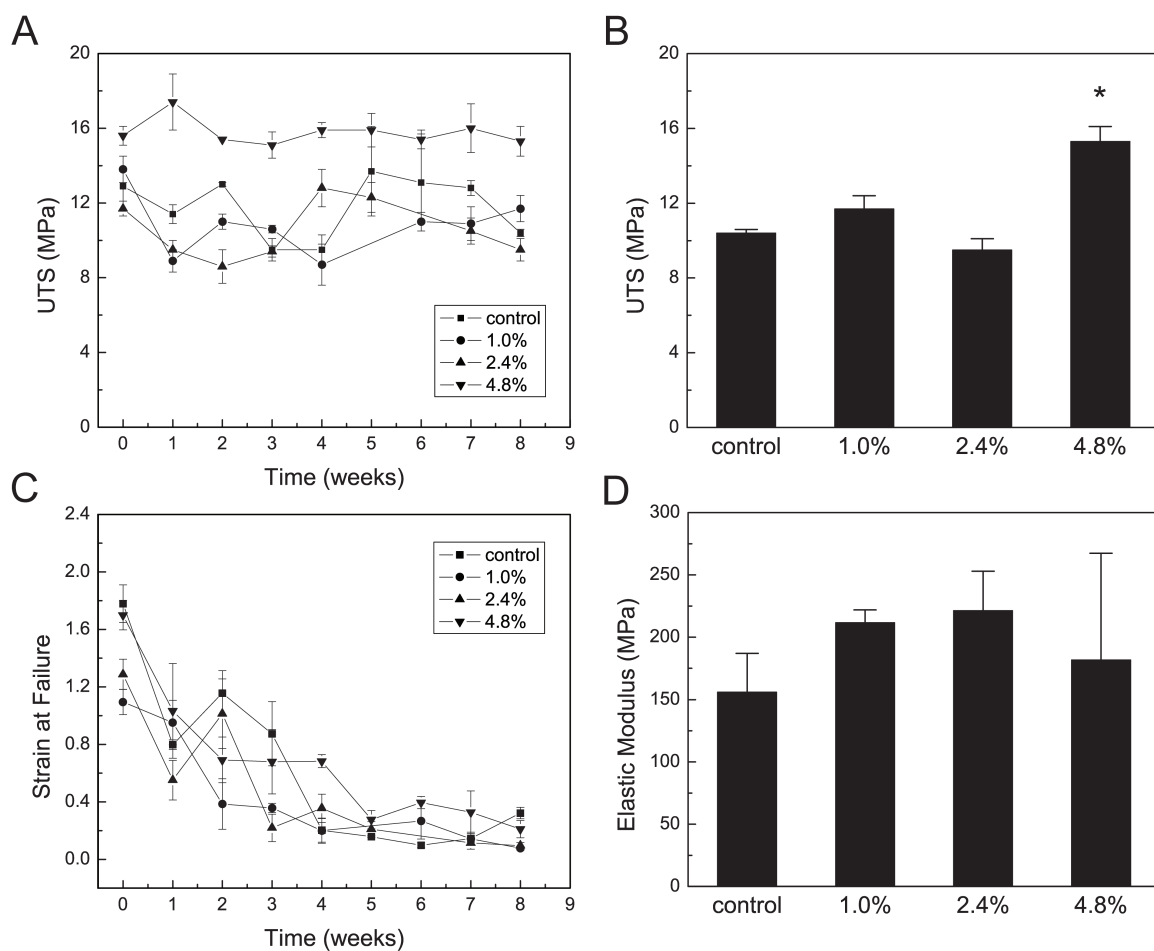


Figure 3.9: Mechanical characteristics of degraded wet spun microfibers. (A) Tensile loss profiles of PLLA microfibers as a function of incubation time, (B) comparison of tensile strength for DXM-loaded and control microfibers after eight-week incubation, (C) strain at failure of incubated microfibers over time, and (D) comparison of elastic moduli at eight-week incubation. Mean $\pm$ SEM are presented, * $p < 0.05$ by ANOVA.

Chapter 4

Effects of protein molecular weight on the intrinsic material properties and release kinetics of wet spun polymeric microfiber delivery systems

Wet spun microfibers have great potential for the design of multifunctional controlled release scaffolds. Understanding aspects of drug delivery and mechanical strength, specific to protein molecular weight, may aid in the optimization and development of wet spun fiber platforms. This study investigated the intrinsic material properties and release kinetics of poly(L-lactic acid) (PLLA) and poly(lactic-co-glycolic acid) (PLGA) wet spun microfibers encapsulating proteins with varying molecular weights. A cryogenic emulsion technique developed in our lab was used to encapsulate insulin (5.8 kDa), lysozyme (14.3 kDa), and bovine serum albumin (BSA, 66.0 kDa) within wet spun microfibers (~100 μm). Protein loading was found to significantly influence mechanical strength and drug release kinetics of PLGA and PLLA microfibers in a molecular weight-dependent manner. BSA encapsulation resulted in the most significant decrease in strength and ductility for both PLGA and PLLA microfibers. Interestingly,

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BSA-loaded PLGA microfibers had a two-fold increase (8 ± 2 MPa to 16 ± 1 MPa) in tensile strength and four-fold increase (3 ± 1 % to 12 ± 6 %) in elongation until failure in comparison to PLLA microfibers. PLGA and PLLA microfibers exhibited prolonged protein release up to 63 days *in vitro*. Further analysis with the Korsmeyer-Peppas kinetic model determined the mechanism of protein release was dependent on Fickian diffusion. These results emphasize the critical role protein molecular weight has on the properties of wet spun filaments, highlighting the importance of designing small molecular analogues to replace growth factors with large molecular weights.

4.1 Introduction

Micro- and nanoscale fibers have gained considerable interest in the field of biomedical engineering [1-5]. In particular, microfibers prepared by wet spinning have been used as drug delivery devices and tissue engineering scaffolds due to their high surface area-to-volume ratios for efficient drug release and ability to be manipulated into a variety of complex macro-level scaffolds. To this end, the use of wet spun fibers as vehicles for drug delivery and biocompatible scaffolds for the assembly of regenerating tissue have been extensively studied. Controlled release of drug molecules has been achieved by encapsulating therapeutics within the polymer matrices of wet spun fibers [6-10]. Scaffolds made from a number of wet spun polymers, such as poly(lactic-*co*-glycolic acid) (PLGA), poly(L-lactic acid) (PLLA), polycaprolactone (PCL), and chitosan have also demonstrated the effectiveness of wet spun microfilaments as morphological guides for tissue regeneration [4, 11-14].

Recently, controlled-release technologies and tissue engineering strategies have been combined for the regeneration of tissues, which require a complex sequence of biological cues and structural support [15-18]. Wet spun microfibers have great potential for the design of multifunctional polymeric systems. To date, the majority of research involving wet spun fibers focuses on the release of therapeutics or biocompatibility and tissue

regeneration capabilities of 3D scaffolds. Little is known about the effects of protein encapsulation on the material properties of wet spun fibers. Moreover, studies that have evaluated the mechanical properties of drug-eluting wet spun microfilaments only focused on small molecules (< 1 kDa) or model proteins of similar molecular weight [8-10, 19]. Textile structures used for ‘next generation’ tissue engineering strategies require structural support and controlled delivery of therapeutics with a wide range of molecular weights. In the past, our lab has shown that protein molecular weight influences drug release kinetics from PLGA microspheres [20]. To the best of our knowledge, this effect has yet to be evaluated in a wet spun fiber-based drug delivery platform.

The mechanical properties of drug-eluting microfibers are very important to their functionality as therapeutic scaffolds in the clinical arena. Mechanical properties of scaffold substrates have been shown to affect cell differentiation, proliferation, and migration [21, 22]. Mechanical strength can also alter host tissue integration; ‘mismatched’ material properties can effect the maturation of regenerated tissues [18]. For clinical applications, the structural integrity of therapeutic implants is critical for surgical handling and implantation. Understanding the effect of protein encapsulation on the material properties of wet spun microfilaments may help predict the properties of macro-level scaffolds to optimize the function and growth of specific cell types. This paper contributes to the fundamental knowledge of the effects of protein molecular weight and encapsulation on the intrinsic material properties and release kinetics of wet spun microfilaments. Three proteins of varying molecular weight: insulin (5.8 kDa), lysozyme (14.3 kDa), and bovine serum albumin (66.0 kDa), were encapsulated into PLGA and PLLA microfibers by phase inversion, similar to previous studies from our lab involving microspheres [20]. Mechanical testing and thermal analysis were used to determine the effect of protein encapsulation and molecular weight on the material properties of wet spun microfibers. The influence of protein molecular weight on the release kinetics and mechanism of release was also characterized.

4.2 Materials and methods

4.2.1 Materials

Poly(L-lactic acid) (PLLA, i.v. = 1.04 dL/g in CHCl_3) (Lactel Biodegradable Polymers) and poly(D,L-lactic-*co*-glycolic acid) 75:25 ester terminated (PLGA, i.v. = 0.55-0.75 dL/g in CHCl_3) (Durect) were used in the fabrication of wet spun microfibers. Bovine serum albumin (Sigma), lysozyme (Sigma) and bovine zinc insulin (Gibco) were used as model proteins for this study. Dichloromethane (Fisher) and petroleum ether (Fisher) were the solvent and nonsolvent used for wet spinning. Sorbitan trioleate (Span 85, HLB 1.8) (Sigma) was used in PLGA microfiber formulations. The micro BCA protein assay reagent kit used to detect protein concentration was used according to the manufacturer (Fisher).

4.2.2 Microfiber fabrication

Spin dope solutions were prepared using a modified cryogenic emulsion technique developed in our lab [23]. PLGA and PLLA microfibers were loaded with one of three proteins: bovine zinc insulin (INS, 5.8 kDa), lysozyme (LZ, 14.3 kDa), and bovine serum albumin (BSA, 66.0 kDa). Briefly, 0.5 mL of protein in ultra-pure water (20 mg mL^{-1}) and 10 mL of polymer in DCM (50 mg mL^{-1}) were combined for an aqueous to organic phase ratio of 1:20. This two-phase system was then vortexed for 60 s, immediately frozen in liquid nitrogen, and lyophilized for 48 h, to yield micro / nanonized proteins distributed in a continuous phase of polymer. The dried polymer and protein product was reconstituted in 2.5 mL DCM at a concentration of 200 mg mL^{-1} and placed into a gas-tight glass syringe fitted with a 22-gauge spinneret. A syringe pump was used to extrude the spin dope solution (0.02-0.06 mL min^{-1}) into petroleum ether at a solvent to nonsolvent ratio of 1:400, which resulted in the continuous formation of PLGA and PLLA monofilaments. PLGA formulations required 50 μL of

Span 85 to prevent gelation around the spinneret during extrusion. precipitate into a fiber. Extruded microfibers were collected from the coagulation bath after all of the spin dope was extruded (~1.5 h residence time). Blank microfibers were also fabricated and used as controls. Duplicate batches of each formulation were made to ensure the reproducibility and uniformity of release profiles.

4.2.3 Scanning electron microscopy (SEM)

Lyophilized microfibers were mounted on adhesive metal stubs and sputter-coated with a 50–100 Å layer of gold-palladium (Emitech). Samples were viewed with a Hitachi S-2700 scanning electron microscope using an accelerating voltage of 8 kV. Micrographs were taken using a Quartz PCI digital imaging system. To determine average cross-sectional area, microfibers were arranged into bundles onto paraffin film and rolled laterally into cylindrical tubes and orthogonally cut into thin discs. Five fields of view (15–22 filaments) of cross-sectioned microfibers were captured at 350x magnification and analyzed using ImageJ software (NIH).

4.2.4 Differential scanning calorimetry (DSC)

DSC measurements of blank and protein-loaded microfibers after fabrication (day 0) and incubation (day 63) were carried using a DSC-7 (Perkin Elmer) equipped with an Intracooler 2 intercooling system. Samples were subjected to: cooling to -25°C, heating to 250°C at 10°C min⁻¹, cooling to -25°C at 10°C min⁻¹, and then reheating to the upper limit again at the initial rate. The crystallinity (X_c) of PLLA microfibers was calculated using

$$X_c = \frac{\Delta H_m}{93.7} \times 100 \quad (\text{equation 4.1})$$

where 93.7 J g⁻¹ is the specific heat of melting of a 100% crystalline PLLA as reported

in the literature [24].

4.2.5 X-ray diffraction (XRD)

The structural properties of PLLA formulations at time zero and 63 days after degradation were also determined using an automated X-ray diffractometer (Siemens Diffraktometer D5000) with a Cu K α ($\lambda = 1.54\text{\AA}$) radiation. The diffraction angles (2θ) ranged from 6° to 60° with sampling intervals of 0.02° s^{-1} . Diffraction signal intensity was monitored and processed using DiffracPlus Software (Bruker AXS).

4.2.6 Mechanical testing

Uniaxial tensile tests were conducted using a materials testing system (Instron Model 4442) in accordance to the United States Pharmacopeia absorbable suture testing standards [25]. Briefly, microfibers and yarns were secured to a paper frame (25 mm x 25 mm) and loaded into the crosshead clamps of the machine. Prior to loading, the sides of the paper template were cut leaving the sample intact. An elongation rate of 50 mm min^{-1} was applied until failure. The resulting load-displacement data collected by the digital acquisition system was converted to stress-strain data to calculate the ultimate tensile strength (UTS), percent strain to failure, and elastic modulus. The mechanical properties of microfibers were compared in SPSS v.19 (Chicago, IL) using a one-way analysis of variance (one-way ANOVA) test. *Post hoc* analyses for multiple comparisons were carried out using the Tukey multiple comparisons test. A p value of less than 0.05 was considered statistically significant.

4.2.7 Protein release studies

To determine protein release kinetics, triplicate samples (10 mg) from each batch were suspended in 1.5 mL of PBS (pH 7.4) in capped microcentrifuge tubes and incubated at 37°C. At each time point, 1 mL of the releasate was removed by pipette and replaced with fresh PBS. The protein concentration of releasates and corresponding standards in PBS were determined using a micro BCA assay kit. Samples and corresponding standards were read by UV absorption at 562 nm. *In vitro* experiments were done in triplicate from duplicate batches of each formulation. The release profiles are expressed as the cumulative percent release of each formulation normalized to the amount of encapsulated protein. Microfibers from each formulation that were immersed in 0.1 *N* NaOH (Sigma) and incubated at 37°C until dissolved. The encapsulation efficiency was calculated as the actual amount of protein detected with the micro BCA assay, as previously described, relative to the theoretical amount of protein added to spin dope solutions prior to wet spinning.

4.2.8 Release data analysis

The data obtained from *in vitro* protein release was fitted to the Korsmeyer-Peppas kinetic model to determine the mechanism of release. Korsmeyer et al. [26] derived a simple relationship to describe diffusion based drug release from a polymeric system given by

$$\frac{M_t}{M_\infty} = kt^n \quad (\text{equation 4.2})$$

where $\frac{M_t}{M_\infty}$ is a fraction of drug released at time t , k is the release rate constant, and n is the release exponent. In this model, the n value is used to characterize the release mechanism. For a cylindrical matrix, $n \leq 0.45$ corresponds to Fickian diffusion, $0.45 < n < 0.89$ to non-Fickian transport, $n = 0.89$ to case II (relaxational) transport, and n

> 0.89 to super case II transport. Data were plotted as log cumulative percentage drug release *versus* log time for the first 60% of drug released.

4.2.9 Fourier transform-infrared (FT-IR) spectroscopy analysis

FT-IR analysis was performed after microfiber fabrication and 63 days of incubation using a Perkin Elmer (Wellesley, MA) Spectrum Once B spectrophotometer with a zinc-selenide (Zn-SE) universal attenuated total reflectance (ATR) attachments. Between 2–3 mg of microfibers were analyzed in the region between 4000–650 cm^{-1} with a resolution of 2 cm^{-1} .

4.3 Results

4.3.1 Microfiber morphology

Scanning electron microscopy was used to study cross-sectional and surface morphology of wet spun microfibers. All formulations underwent phase inversion roughly 2-4 cm from the spinneret tip, forming a continuous solid white monofilament. Microfibers were consistently sized, with similar cross-sectional area, porosity, and porous interconnectivity. Blank and protein-loaded PLGA microfibers had lobed ‘dog-bone’ shape with an average height of 46 μm and width of 105 μm (**Figure 4.1**). Blank and protein-loaded PLLA formulations displayed lobed ‘kidney’ shape with an average height of 50 μm and width of 102 μm (**Figure 4.2**).

4.3.2 Thermal analysis

Differential scanning calorimetry was used to study the thermal properties of PLGA and PLLA microfibers after fabrication (day 0) and the incubation period (day 63). **Table 4.1** summarizes the thermal transitions of wet spun microfibers from the first DSC heating scan. The glass transition temperature (T_g) of blank PLGA microfibers was 40.7°C. The T_g of microfibers slightly increased (1.3-3.7°C) with protein loading. After 63-day incubation, all PLGA formulations, including blank microfibers exhibited an increase in T_g in the range of 2.4-5.0°C.

Blank PLLA microfibers had a T_g of 42.1°C, with a T_m of 173.0°C, and relative crystallinity of 26.2%. The T_g of INS and BSA-loaded microfibers slightly decreased by 1.3°C and 1.4°C; LZ microfibers were similar to the control. A minor increase in relative crystallinity (2.6-3.2%) was observed with INS and LZ-loaded PLLA microfibers in relationship to blank PLLA microfibers. BSA-loaded formulations had the lowest relative crystallinity of 25.9% and T_g of 40.7°C. In contrast to PLGA formulations, incubated PLLA (63 day) microfibers had a substantial increase in T_g in the range of 16.1 to 18.5°C. PLLA microfibers also had a slight increase in percent relative crystallinity; blank microfibers and BSA-loaded microfibers exhibited the greatest increase in percent relative crystallinity of 4.3% and 4.4%, respectively. The T_m for all PLLA formulations remained consistent throughout the incubation period.

4.3.3 XRD analysis

XRD experiments were used to determine the crystalline structure of PLLA microfibers. X-ray scattering patterns of blank and protein-loaded microfibers before (day 0) and after incubation (63 days) are presented in **Figure 4.3**. For blank PLLA microfibers, two small peaks (13.0° and 17.6°) and one large peak (24.4°) were observed. This large diffraction peak diminished with protein loading (**Figure 4.3A**). After incubation in

PBS at 37°C for 63 days, the diffraction peak at 24.4° disappeared and a new diffraction peak appeared between 16-17°, which was significantly enhanced for protein-loaded samples (**Figure 4.3B**).

4.3.4 Mechanical properties

To determine the effect of protein loading and molecular weight on the mechanical properties of PLGA and PLLA wet spun microfibers, samples were loaded under uniaxial tension until failure. Since microfibers were non-circular, the average cross-sectional areas were calculated from SEM images for mechanical analyses. The mechanical strength of blank PLLA microfibers was greater than blank PLGA microfibers (**Figure 4.4**). Blank PLLA microfibers had an average load to failure of 176.4 ± 3.8 mN and ultimate tensile strength of 43.2 ± 0.9 MPa, whereas blank PLGA microfibers had an average load to failure of 118.6 ± 14.0 mN and ultimate tensile strength of 32.7 ± 3.8 MPa (**Figure 4.4A, B**). Significant variations in the strain to failure and elastic modulus were also observed among blank formulations. PLGA microfibers had a strain to failure of $12 \pm 6\%$ in comparison to PLLA microfibers with a strain to failure of $3 \pm 1\%$ (**Figure 4.4C**). PLGA microfibers had an average elastic modulus of 569.7 ± 61.7 MPa whereas PLLA microfibers had an average elastic modulus of 822.4 ± 93.3 MPa (**Figure 4.4D**).

Protein encapsulation notably altered the material properties of both PLGA and PLLA microfibers. Protein-loaded microfibers within the same polymer type had significantly lower ($p < 0.05$) load to failure and ultimate tensile strength in comparison to blank controls (**Figure 4.4A, B**). Among protein-loaded formulations, BSA-loaded PLGA and PLLA microfibers possessed significantly lower ($p < 0.05$) load to failure and ultimate tensile strength in comparison to INS and LZ-loaded formulations. However, no statistical differences in load to failure and ultimate tensile strength were observed between INS and LZ-loaded PLGA formulations. A linear relationship could be

established between the decrease in load to failure and ultimate tensile strength with increasing protein molecular weight for PLGA ($R^2 = 0.8967, 0.9448$) and PLLA ($R^2 = 0.9906, 0.9995$) formulations, respectively (data not shown).

Significant differences in strain at failure were also observed within PLGA and PLLA formulations (**Figure 4.4C**). The failure extensions of protein-loaded PLGA microfibers were statistically lower than blank PLGA microfibers ($p = 0.000$). LZ and BSA-loaded PLLA microfibers also had statistically lower elongation until failure in comparison to blank PLLA microfibers. Interestingly, INS-loading did not significantly decrease the ductility of PLLA microfibers ($p = 0.659$). An exponential relationship was found between decreasing strain to failure and increasing molecular weight of the protein encapsulated in PLGA ($R^2 = 0.9880$) and PLLA ($R^2 = 0.9800$) microfibers (data not shown). No statistical differences in elastic moduli were observed among PLGA formulations (**Figure 4.4D**). However, BSA-loaded PLLA microfibers had significantly lower elastic modulus in comparison to blank, INS-loaded and LZ-loaded formulations.

4.3.5 Microencapsulation efficiencies

Actual loading and protein encapsulation efficiencies are listed in **Table 4.2**. PLGA formulations had lower average loading efficiencies of $38.3 \pm 6.7\%$ in comparison to PLLA efficiencies of $68.0 \pm 5.7\%$. Protein loss can be attributed to protein adsorption to conical tubes during the micronization process and differences spin dope precipitation strength during phase inversion. PLLA solutions precipitated visibly faster than PLGA solutions, which could have accounted for increased encapsulation efficiency among PLLA formulations. Protein loss could be reduced in the scale-up to larger batch sizes.

4.3.6 Protein release kinetics and analysis

Three phases were observed during release from microfiber formulations (**Table 4.2**). During the first phase, protein release was fastest for INS, followed by LZ and BSA for PLGA formulations (**Figure 4.5A**). Similar patterns were observed for PLLA formulations, but at significantly lower cumulative release percentages (**Figure 4.5B**). The duration of the first phase was characterized by a typical diffusion profile and was found to be dependent on protein molecular weight. For PLGA microfibers, this phase lasted for 14 days for INS and 21 days for LZ and BSA. The first phase of INS, LZ and BSA release from PLLA fibers was 14 days. A second phase, characterized by slow, linear release was also observed for all PLGA and PLLA formulations. The duration of this phase for INS was 28 days and 21 days for PLGA and PLLA formulations, respectively. LZ and BSA PLGA formulations had a second phase of 42 days; this phase was 49 days for PLLA formulations. A third phase with additional protein release was only observed with INS formulations, which lasted 21 days for PLGA and 28 days for PLLA formulations.

To determine the mechanism of protein release from microfibers, *in vitro* release data was fitted to the Korsmeyer-Peppas kinetic model. The regression coefficient (R^2) and release constant (k) values from the release data for each formulation were calculated as shown in **Table 4.3**. The regression coefficients for Korsmeyer-Peppas plots ranged from 0.9684 to 0.9865, showing good agreement between the experimental drug release and kinetic modeling. The release constant values were also found to decrease with increased protein molecular weight, consistent with release profiles. Moreover, the n values calculated for all protein formulations were below 0.45, describing the release mechanism as typical Fickian diffusion.

4.3.7 FT-IR analysis

FT-IR spectra were taken at the beginning and end points of the release study to characterize the relative degradation of incubated microfibers. **Figure 4.6** shows the average spectra from PLGA and PLLA microfiber formulations after fabrication (0 days) and 63 days of incubation. The FT-IR spectra remained consistent throughout our study. The three absorption peaks characteristic of the ester bonds are the ester aliphatic C=O stretch at 1750 cm^{-1} , aliphatic ester C–O stretch at 1260 cm^{-1} , and the $\nu(\text{C–C})$ mode of the C–COO of the polymer chain at 860 cm^{-1} . None of these peaks diminished after 63 days of incubation. There was also no emergence of the peaks indicative of lactic and glycolic acid; the carboxylic acid $\nu(\text{C=O})$ stretch at 1700 cm^{-1} and the wide $\nu(\text{O–H})$ bend at 3200 cm^{-1} .

4.4 Discussion

Wet spinning is a well-established and versatile method for fabricating polymeric microfibers. PLGA and PLLA were chosen for this study because they are commonly used biodegradable materials and have FDA approval in many medical applications. Three proteins of varying molecular weights, insulin (5.8 kDa), lysozyme (14.3 kDa), and bovine serum albumin (66.0 kDa) were encapsulated in PLGA and PLLA to study how protein molecular weight and polymer type affect the release kinetics and intrinsic material properties of wet spun microfibers. The optimal protein loading of 2% (w/w) was determined based on the maximum amount of BSA that could be loaded into 20% (w/v) polymer concentrations without disrupting the continuous formation of fibers. Therapeutics with molecular weights higher than BSA could be incorporated into fibers by increasing the concentration of the spin dope, increasing the molecular weight of the polymer, or by decreasing the theoretical loading of the protein. Similarly, other

polymers could easily be fabricated into wet spun microfibers by selecting the appropriate solvents and nonsolvents.

All fibers prepared from cryogenic emulsions had non-circular skin-core structure consistent with the wet extrusion process. Fiber shape can be explained by solvent and nonsolvent counter-diffusion. If the rate of solvent diffusing out is higher than the rate of nonsolvent diffusing in, the fiber structure collapses and non-circular shapes such as lobed ‘kidney’ and ‘dog bone’ are formed [27]. Rapid surface coagulation during phase inversion also leads to the entrapment of solvent and nonsolvent within the precipitating microfilament [28]. Porous structure is formed once solvent and nonsolvent are evaporated after microfilament solidification. PLGA and PLLA microfibers from this work had significantly less voids than poly(L,D-lactic acid) fibers prepared from water-in-oil (W/O) emulsions by Rissanen et al. [19]. In the previous study, pores were attributed to air bubbles from emulsion formation and rapid phase separation during filament precipitation in the spin bath. Our cryogenic emulsion process reduces the potential for large voids by removing the water phase from the primary W/O spin dope. Gao et al. also reduced void formation by encapsulating drugs as solid particles within wet spun microfibers [6]. However, in contrast to our technique, hydrophilic drugs encapsulated in the previous example were not micro / nanonized and thus resulted in large particles imbedded within the fibers and on the surfaces of fibers.

Thermal analyses showed that the glass transition temperatures of PLGA formulations did not substantially decrease with protein loading, nor did the relative crystallinity of PLLA formulations. These results, when taken together, indicate the long duration (~1.5 h) of the fibers in the nonsolvent bath may have led to solvent-induced crystallization (SINC). We have evidence that shorter residence time (10-15 s) in the nonsolvent bath resulted in lower crystallinity [29]. Of particular interest is the fact that all samples from this study, including blank fibers, demonstrated high relative crystallinity and that SINC is persistent despite the presence of the encapsulated protein. Evidence of further crystallization with incubation was also evident with the increase in glass transition

temperature observed among all formulations. We believe the increase in glass transition temperature could be due to increased amorphous chain mobility at the incubation temperature (37°C) and potential formation of ordered structures. Re-ordering of shorter oligomers and strong secondary interactions between proteins and polymers could have further contributed to this effect, as reflected in the formation of a new x-ray diffraction peak around 16.5° among protein-loaded formulations.

As well, the molecular weight of the protein and polymer structure also influenced the physical properties of wet spun microfilaments. While both polymers exhibited significantly reduced tensile strength with increasing protein molecular weight, PLLA microfibers loaded with BSA had the greatest tensile loss. This may be explained by the differences in polymer structure between PLGA and PLLA. PLLA is a semi-crystalline polymer whereas PLGA is amorphous. In general, the amorphous regions of a polymer require less force to deform in comparison to the crystalline regions. At high protein molecular weight loading with BSA, the material properties of PLGA and PLLA microfibers were significantly weakened possibly due to protein particles acting as material defects within the polymer lattice during elastic deformation. However, BSA-PLGA microfibers had higher tensile strength and elongation until failure than PLLA. This suggests the amorphous content of PLGA appeared to not only modulate the release of protein, but also reduce the tensile loss and embrittlement of BSA-loaded microfibers relative to BSA-loaded PLLA formulations. Differences between amorphous PLGA and semi-crystalline PLLA are also reflected in elastic moduli. PLGA microfibers had similar resistance to deformation regardless of protein loading or protein molecular weight. PLLA is semi-crystalline and so therefore low molecular weight protein loading provided slightly increased resistance to deformation likely due to protein particles reinforcing the amorphous regions of the polymer. Protein-polymer interactions at the molecular level could also affect the physical properties of the microfiber. Strong secondary interactions between proteins and polymers, such as hydrogen bond formation and ionic interactions, could help to maintain the material properties of polymeric

microfibers with INS and LZ loading. Other researchers have shown the encapsulation of small molecules (< 1 kDa) decreases wet spun fiber strength and ductility [8-10]. This study evaluated multiple proteins of varying molecular weight and shows protein molecular weight plays a critical role in the tensile strength of polymeric wet spun microfibers.

Protein release from all wet spun microfibers was found to increase with decreasing protein molecular weight. This data suggests the amount of protein released is controlled by water penetration, swelling, and to some extent, degradation. The fact that we see a third phase in the release of INS from PLGA and PLLA formulations implies some degradation is occurring. In the degradation of polyesters, random cleavage of ester linkages along the polymer backbone breaks long polymer chains into short fragments that may not at first be water-soluble. A reduction in the molecular weight increases hydrophilicity, which may be why we are seeing additional release of INS with little polymer degradation as judged by DSC and FT-IR analyses. These findings are consistent with the slow degradation of wet spun aliphatic polyesters with similar molecular weights [30, 31].

It is also important to note that the chemical structure of proteins and protein-protein and protein-polymer interactions may also have affected *in vitro* release kinetics. For example, the isoelectric point (pI), or the pH at which a molecule carries no charge, for INS, LZ, and BSA is 5.3, 10.7, and 4.8, respectively. Throughout our studies, the pH of the buffer solution maintained a pH from 6.0 to 7.4 (data not shown). When the $\text{pH} < \text{pI}$, molecules carry a positive net charge, while when the $\text{pH} > \text{pI}$, molecules carry a negative net charge. Thus, in our study, INS and BSA were negatively charged, and LZ was positively charged. Since most polymers are slightly negatively charged, ionic interactions between protein-polymer and protein-protein could contribute to protein release.

4.5 Conclusions

Our findings indicate the *in vitro* release kinetics and mechanical properties of wet spun polymeric microfibers are heavily dependent on protein molecular weight, highlighting the importance of designing small molecular analogues to replace growth factors with large molecular weights for the development of multifunctional fiber delivery systems. Perhaps the most interesting observation is that release of a hydrophilic protein as small as INS (5.8 kDa) lasted for the entirety of the experiments (63 days) with nearly 80% drug still remaining within PLLA microfibers. The slow, continuous release of small proteins may constitute a clinically relevant therapeutic approach for the regeneration of tissues that require long-term growth factor delivery. In addition, characterization of the material properties showed that the strength and elongation of wet spun fibers was material-specific. The amorphous content of PLGA appeared to modulate the release of protein and reduce the tensile loss and embrittlement of BSA-loaded microfibers relative to BSA-loaded PLLA formulations; this suggests semi-crystalline polymers, such as PLLA, may be favorable for the encapsulation of small molecular weight proteins (< 66.0 kDa) and that amorphous polymers, such as PLGA, may be optimal for the encapsulation of higher molecular weight proteins in the design of multifunctional drug-eluting fibers. The amorphous content of PLGA could also be tailored to extend the release of protein and to increase the tensile strength of fibers by altering the lactic acid to glycolic acid ratio. The wet spun microfibers prepared by cryogenic emulsion from this study could be used as a platform for the optimization and design of future fiber-based delivery systems.

4.6 References

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4.7 Tables

Table 4.1: Summary of the thermal properties from the first DSC heating scan of wet spun microfibers after fabrication (0 days) and incubation (63 days). Data are expressed as mean $\pm$ SD ($n = 2$).

Microfiber	Time	T_g ($^{\circ}\text{C}$)	T_m ($^{\circ}\text{C}$)	ΔH_m (J g^{-1})	X_c (%)
<i>(A) PLGA</i>					
Blank	t = 0	40.7 ± 0.5	—	—	—
	t = 63	45.3 ± 1.1	—	—	—
INS	t = 0	44.4 ± 3.8	—	—	—
	t = 63	46.8 ± 0.6	—	—	—
LZ	t = 0	42.4 ± 1.9	—	—	—
	t = 63	47.4 ± 0.7	—	—	—
BSA	t = 0	42.0 ± 1.5	—	—	—
	t = 63	46.3 ± 0.4	—	—	—
<i>(B) PLLA</i>					
Blank	t = 0	42.1 ± 2.2	173.0 ± 0.1	24.6 ± 2.4	26.2 ± 2.6
	t = 63	58.2 ± 0.7	173.3 ± 1.5	28.6 ± 1.0	30.5 ± 1.1
INS	t = 0	40.8 ± 0.4	173.0 ± 1.5	27.5 ± 0.4	29.4 ± 0.4
	t = 63	59.1 ± 0.5	172.0 ± 0.6	27.5 ± 0.3	29.3 ± 0.3
LZ	t = 0	42.4 ± 2.5	173.0 ± 1.4	27.0 ± 0.5	28.8 ± 0.5
	t = 63	58.9 ± 0.7	172.0 ± 0.0	28.4 ± 1.7	30.3 ± 1.8
BSA	t = 0	40.7 ± 0.6	173.3 ± 1.3	24.3 ± 0.6	25.9 ± 0.6
	t = 63	59.2 ± 0.5	171.3 ± 0.6	28.4 ± 0.3	30.3 ± 0.3

T_g , glass transition temperature

T_m , melting temperature

ΔH_m , endothermic enthalpy of the melting peak

X_c , crystallinity degree

Table 4.2: Summary of protein release data from 2% (w/w) loaded microfibers

Microfiber	Molecular weight (kDa)	Actual loading (%)	Encapsulation efficiency (%)	First phase duration and release	Second (lag) phase duration and release	Third phase duration and release	Total released (%)
<i>(A) PLGA</i>							
INS	5.8	0.83	41.6	14 days 57.9%	14–42 days 8.7%	42–63 days 14.7%	81.3
LZ	14.3	0.85	42.7	21 days 40.4%	21–63 days 7.1%	None	47.5
BSA	66.0	0.61	30.5	21 days 28.1%	21–63 days 12.3%	None	40.5
<i>(B) PLLA</i>							
INS	5.8	1.46	73.0	14 days 13.4%	14–35 days 0.7%	35–63 days 4.4%	18.5
LZ	14.3	1.39	69.2	14 days 4.5%	14–63 days 3.9%	None	8.4
BSA	66.0	1.24	61.8	14 days 3.4%	14–63 days 1.7%	None	5.1

Table 4.3: Release parameters of protein-loaded microfibers

Microfiber	Korsmeyer-Peppas		
	$k(t^n)$	n	R^2
<i>(A) PLGA</i>			
INS	0.1281	0.2805	0.9865
LZ	0.0461	0.3430	0.9730
BSA	0.0325	0.3419	0.9868
<i>(B) PLLA</i>			
INS	0.0252	0.2708	0.9862
LZ	0.0137	0.3180	0.9653
BSA	0.0131	0.2441	0.9684

4.8 Figures

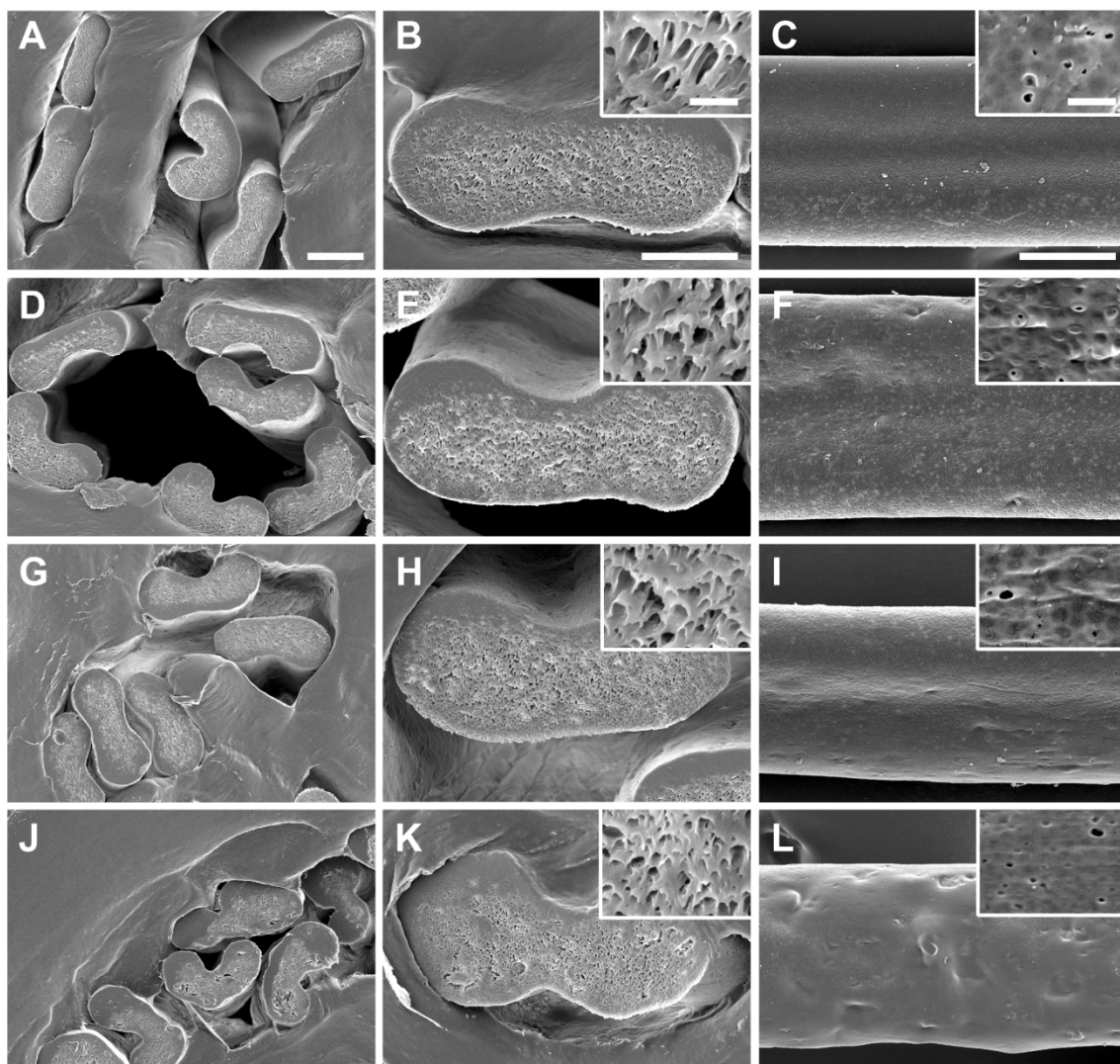


Figure 4.1: Scanning electron micrographs of PLGA microfibers as a function of protein loading. Cross-sectional and surface morphology of blank (A-C), INS-loaded (D-F), LZ-loaded (G-I) and BSA-loaded PLGA microfibers. Scale bars, column I, 50 μm (350x magnification); column II, 30 μm (1kx magnification); column III 50 μm (600x magnification). Insert scale bars are 3 μm (5kx magnification).

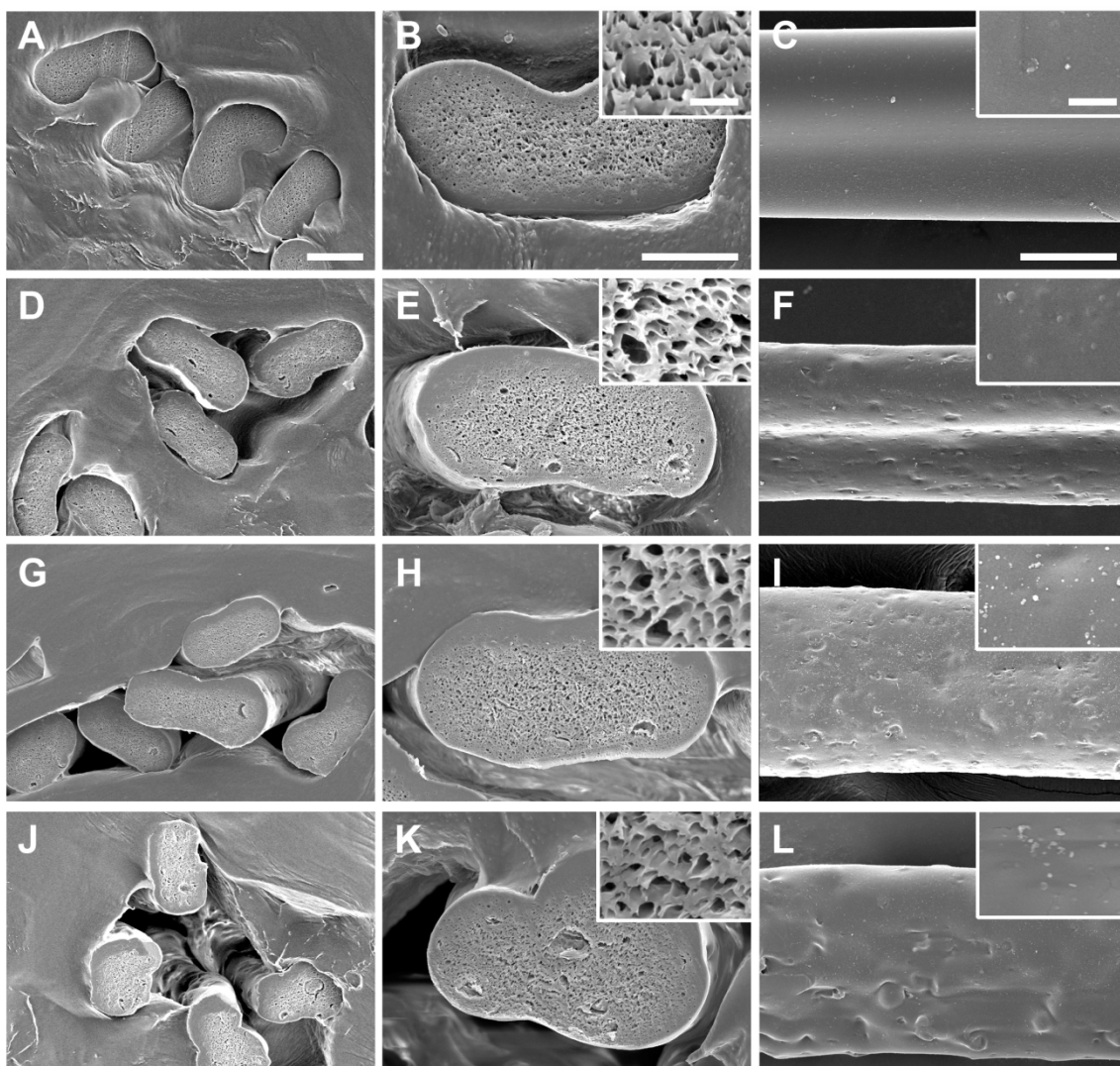


Figure 4.2: Scanning electron micrographs of PLLA microfibers as a function of protein loading. Cross-sectional and surface morphology of blank (A-C), INS-loaded (D-F), LZ-loaded (G-I) and BSA-loaded PLLA microfibers. Scale bars, column I, 50 μm (350x magnification); column II, 30 μm (1kx magnification); column III 50 μm (600x magnification). Insert scale bars are 3 μm (5kx magnification).

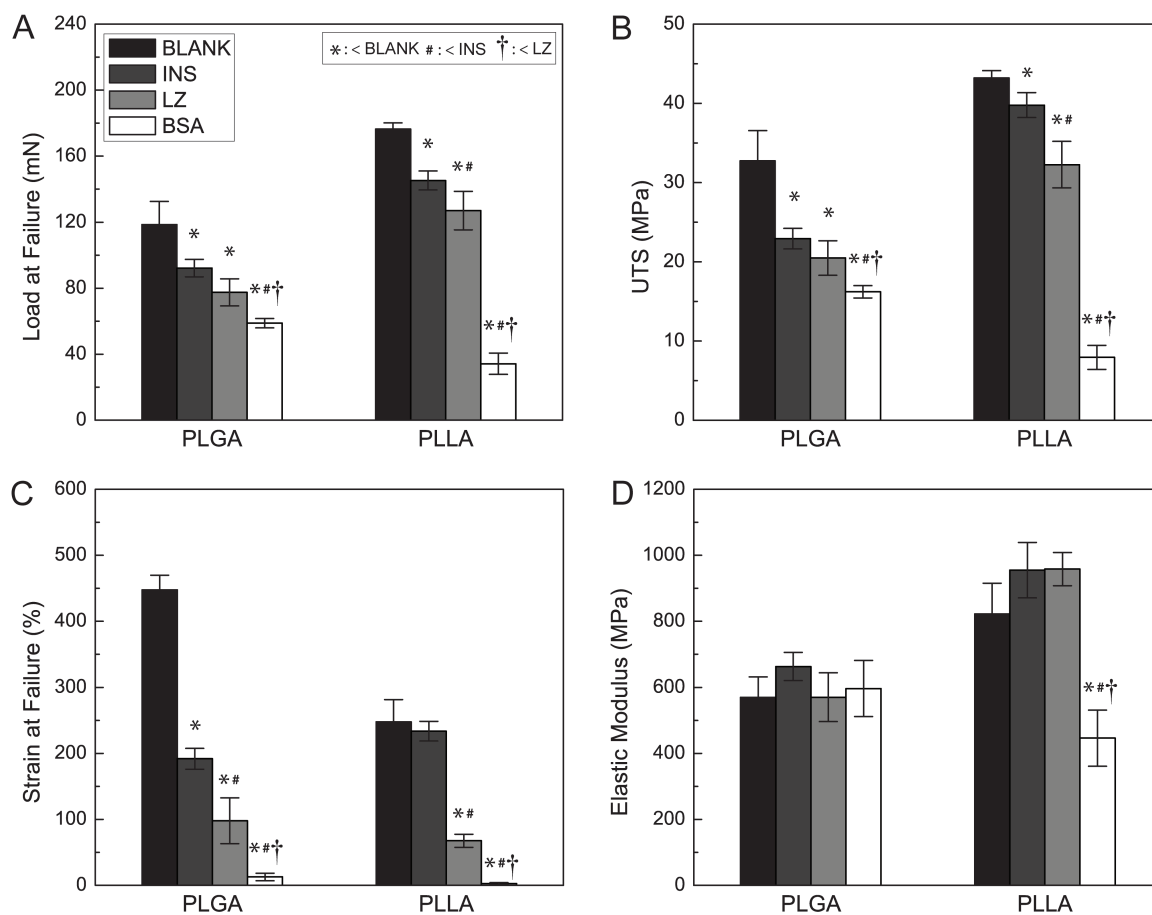


Figure 4.3: Mechanical analyses of wet spun microfibers as a function of protein-loading and polymer type. (A) Load at failure, (B) UTS, (C) strain at failure, and (D) elastic modulus are shown for blank (black), INS-loaded (dark gray), LZ-loaded (gray) and BSA-loaded (white) PLGA and PLLA microfibers. Mean $\pm$ SD are presented ($n = 5$). *, #, †: significant in one-way ANOVA with Tukey *post hoc* multiple comparisons tests.

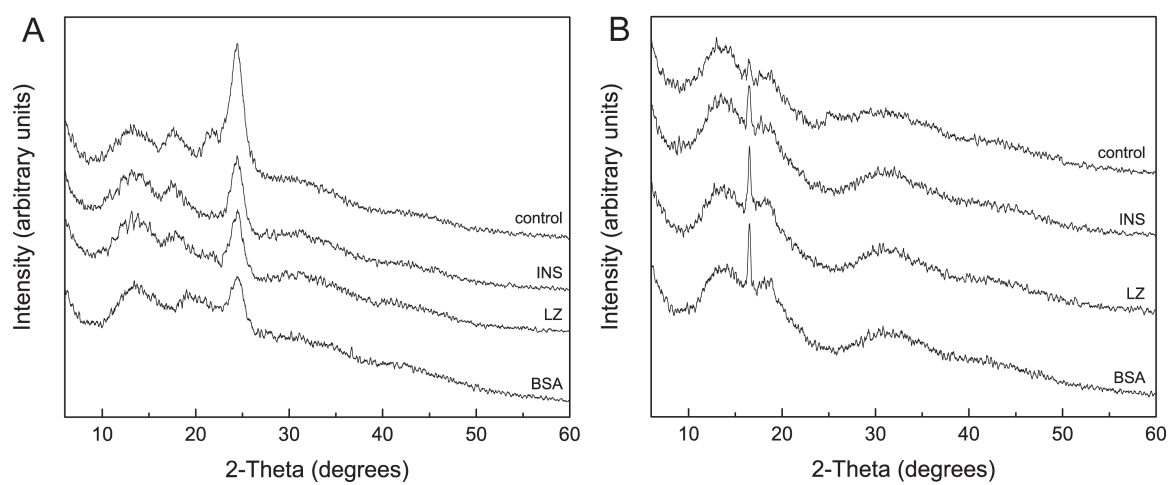


Figure 4.4: XRD diffractograms of blank, INS, LZ, and BSA-loaded PLLA microfibers (A) after fabrication and (B) after incubation for 63 days.

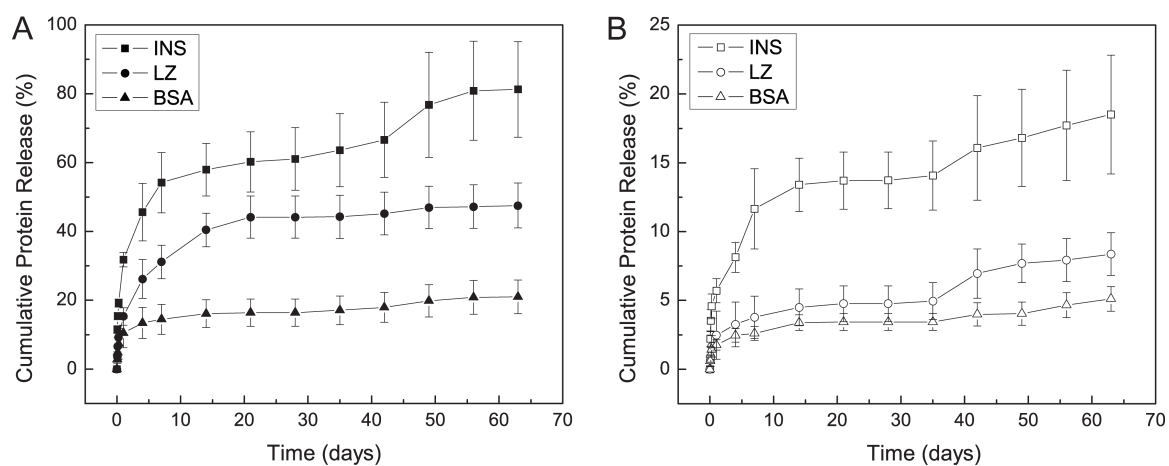


Figure 4.5: Protein release profiles of wet spun (A) PLGA and (B) PLLA microfibers loaded with INS (squares), LZ (circles) and BSA (triangles). Cumulative percent release is presented as mean $\pm$ SD for duplicate batches of each formulation ($n = 6$).

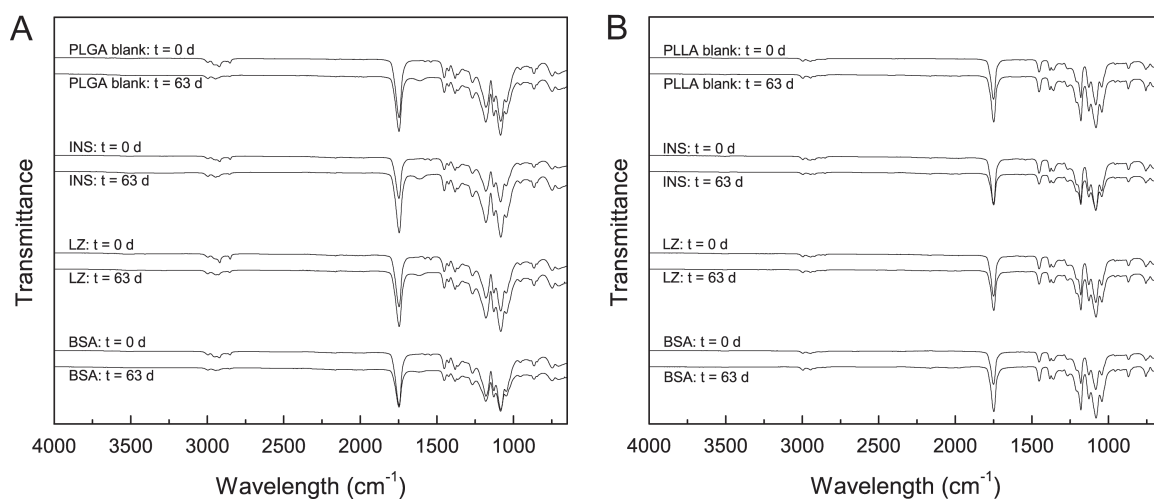


Figure 4.6: FT-IR spectra of (A) PLGA and (B) PLLA formulations after fabrication (0 day) and after incubation (63 day). Each trace represents the average spectra of 16 scans per sample.

Chapter 5

Designing wet spun polymeric monofilaments and multifilament yarns as controlled-release drug delivery systems for biomedical applications

Implanted biomaterials have been shown to elicit a dynamic host inflammatory response that can severely limit the longevity of many biomedical devices. This paper investigates the use of wet spun polymeric fibers as hybrid devices, ones that perform a mechanical function and simultaneously delivery drugs. The functionality of wet spun fibers was evaluated by the release kinetics, mechanical properties, and handling capabilities. Dexamethasone (DXM), a potent anti-inflammatory drug, was encapsulated into wet spun fibers by phase inversion and diverse release profiles were achieved by altering polymer spin dope concentration and composition. Single fibers spun from 10% (w/v) polymer solutions of PLLA, PLGA and PVP displayed a pronounced burst release of (7-20%) after 1-day incubation. In contrast, 20% (w/v) polymer solutions of PLLA and PLGA had delayed release with less than 3% encapsulated drug released after 1-

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day incubation. A ‘Z’ twisting methodology was developed to engineer single microfilaments into multifilament yarns, and the release of DXM was achieved for up to 56 days. DXM eluted from yarns at 1-day and 56-day incubation had the same inhibitory effect on the proliferation of human valve interstitial cells, demonstrating retention of biological activity. Most importantly, DXM was encapsulated within wet spun filaments without compromising mechanical integrity. A five-fold increase in the average load bearing capacity (76 mN to 394 mN) of single, drug-free and drug-loaded filaments was achieved with the formation of 6-ply yarns. This work suggests that wet spun drug-eluting fibers and yarns could be used as building blocks for the design of novel multifunctional anti-inflammatory biomedical implants, such as surgical sutures.

5.1 Introduction

Polymeric fibers have been used to design a variety of medical textiles, such as bioprosthetic valve sewing rings, knitted hernia repair meshes, nerve guidance channels, and, in their simplest form, surgical sutures. Although many of these implants function adequately for years, their integration and long-term performance is severely limited by post-operative inflammation, which can lead to infection, rejection and recurrence [1, 2]. Even single filaments, such as surgical sutures, have been shown to elicit unwanted inflammatory responses [3], contributing to over 500,000 surgical site infections in the US annually [4]. To this end, researchers have sought to improve these devices through tissue engineering strategies as alternative treatments by combining existing biomaterials with natural polymers such as collagen [5, 6]. Interestingly, few researchers have considered using sutures themselves as drug delivery conduits. The majority of studies found in the literature focuses on covalently crosslinking [7, 8] or coating therapeutics [9-11] to existing sutures. However, these systems release drug in a matter of days and consequently limit the sustained release of therapeutics. One approach to achieve controlled, sustained delivery of drugs would be to impregnate or encapsulate

therapeutics within the polymer matrix of the fiber material.

Biodegradable polymeric fibers can be fabricated by a number of methods, the most common of which are melt spinning [12], dry spinning [13], electrospinning [14-16], and wet spinning [17-20]. Melt spinning is currently used to manufacture biodegradable sutures. However, the high processing temperatures used during fabrication are not favorable for the encapsulation of heat sensitive proteins and drugs. Dry spinning may be done at room temperature, but is limited to polymers that dissolve in volatile solvents. Electrospinning is a popular technique used in tissue engineering to fabricate ultrafine nanofibers. Since these fibers offer little structural support as single filaments, recent studies have investigated co-axial spinning for the scale-up of electrospun fibers into micro-scale sutures [21-23]. However, in order to create electrospun microsutures, tens to hundreds of fibers must be grouped together, subsequently increasing the chance of bacterial adhesion and potential for post-operative infection. Wet spinning was selected for this study since it produces micron-sized fibers within the size range of surgical sutures and is most similar to microsphere based drug encapsulation techniques.

We herein propose the use of wet spun fibers for the sustained drug delivery from multifilament yarns for biomedical implants, such as surgical sutures. Polymeric fibers used in the design of such implants must provide mechanical support and simultaneously deliver drug for long periods of time (months). This work investigates the interplay between mechanical strength and drug release kinetics of wet spun polymeric fibers. Our goal was to create 50 to 100 μm fibers capable of physical manipulation so that they could be wound into multifilament yarns for the design of surgically implantable constructs. Dexamethasone (DXM), a synthetic glucocorticoid, was selected as a model drug for this study. DXM has been used commercially in coatings on FDA-approved pacemaker leads [24] and cardiovascular stents [25-27] to suppress chronic inflammatory responses to implants. The local delivery of steroids such as DXM could also eliminate adverse side effects associated with long-term systemic delivery.

5.2 Materials and methods

5.2.1 Materials

Poly(L-lactic acid) of varying molecular weight (PLLA, 1.04 i.v.; PLLA_{0.94}, 0.94 i.v.) and poly(D,L-lactic-*co*-glycolic acid) 75:25 ester terminated (PLGA, 0.55-0.75 i.v.) were purchased from Lactel Biodegradable Polymers (Birmingham, AL) and used as supplied. Polyvinylpyrrolidone (PVP, 10 000 MW) was obtained from Spectrum Chemicals (St. Louis, MO). Reagent grade dichloromethane (DCM), petroleum ether, 2-propanol, and tetrahydrofuran (THF) were purchased from Fischer Scientific (Pittsburgh, PA). Dexamethasone (DXM, 392.5 Da) and all cell culture materials were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise specified.

5.2.2 Fiber spinning

Biodegradable fibers were spun by phase inversion using a wet spinning set-up previously described [28]. Briefly, spin dope solutions of polymer formulations dissolved in DCM were added to a 5 mL pump-controlled syringe fitted with a 22-gauge spinneret. Spin dope solutions were extruded into a nonsolvent coagulation bath resulting in the rapid de-solvation of liquid polymer streams and continuous formation of monofilaments (**Figure 5.1A**). Different spin dope solutions were investigated. For the encapsulation of DXM, 10% and 20% (w/v) solution compositions were fabricated from blends of PLLA, PLGA and PVP in co-solvent mixtures of 6:1 (v/v) DCM to THF. A co-solvent mixture was necessary to increase the solubility of DXM in spin dope solutions. With many spinning parameters possible, fibers were extruded at 0.02–1.2 mL min⁻¹ into petroleum ether, 2-propanol, or mixtures thereof and collected from the spin bath using three distinct methods: i) fibers were either left in the bath throughout the extrusion process (as-spun), ii) drawn from the bath during extrusion (solution-drawn), or iii) removed from the spin bath and wound around metal bobbins under

tension (post-drawn). Each of these processes was developed to determine whether wet spun fibers could be used to design hybrid devices.

5.2.3 Multifilament yarn production

Single fibers were used to produce multifilament yarns. Monofilaments were fabricated by extruding spin dope solutions of PLLA, PLGA, and PVP into a 50:50 (v/v) mixture of 2-propanol to petroleum ether coagulation bath for 1.5 min. The addition of 2-propanol was necessary for some formulations to overcome coagulation around the spinneret tip by slowing the rate of precipitation for the formation of continuous fibers. After fibers cured for 4 min, they were removed from the spin bath and placed into an empty beaker. Samples were untangled and wound around metal bobbins (post-drawn) on a rotating mandrel positioned above the beaker. To create 6-ply yarns, six bobbins, 4 monofilaments of a 10% (w/v) polymer composite formulation ‘A’ and 2 monofilaments of a 20% (w/v) composite formulation ‘B’ were placed on a winding mandrel (pattern B, A, A, A, A, B). Monofilament fibers were grouped together using a surgical clamp and twisted along the longitudinal axis in the ‘Z’ direction (filament inclination from top right to bottom left) to create 30-inch multifilament yarns (**Figure 5.1B**). Yarns were then wound around an empty bobbin and stored at -20°C until further experimentation.

5.2.4 Scanning electron microscopy (SEM)

Representative samples from each formulation were evaluated by SEM. Samples were mounted on adhesive metal stubs and sputter-coated with a 50-100 Å layer of gold-palladium (Emitech, Kent, England). Micrographs were taken with a Hitachi S-2700 (Tokyo, Japan) at an accelerating voltage of 8 kV using a Quartz PCI digital imaging system. To determine the average diameter of microfilaments and yarns, five fields of

view were captured at 100x and 35x, respectively. The average porosity of cross-sectioned fibers was also determined from five fields of view at 1.3kx. Micrographs were analyzed using NIH ImageJ software (Bethesda, MD). Porosity was calculated as the pore area divided by the total cross-sectional area.

5.2.5 Dexamethasone release studies

The polymer composition of DXM-loaded formulations is shown in **Table 5.1**. Monofilaments and multifilament yarns (triplicate samples, 10 mg each) were incubated in 1.4 mL of phosphate buffered saline (PBS) at 37°C. At each time point, 1.0 mL of the releasate was removed and replaced with fresh PBS. Amounts of DXM released from individual fibers and multifilament yarns were quantified by UV absorption at 239 nm. A 96-well quartz plate was used to minimize background at the reading frequency. Drug concentration was determined by comparing absorbance values to standards of known DXM concentration.

5.2.6 Engineering fibers with desired release kinetics from multifilament yarns

The release kinetics of multifilament yarns was predicted from the experimental release of individual monofilaments. For every multifilament yarn, the theoretical release as a function of release time, t was calculated according to

$$\textit{Theoretical Release } (t) = f_A R_A(t) + f_B R_B(t) \quad (\text{equation 5.1})$$

where f is the fraction of fibers from any given formulation relative to the total number of discrete monofilaments included in the yarn, and R is the amount of drug released from individual formulations as a function of release time, t . This equation may be applied to any number of individual formulations twisted to create multifilament drug-eluting yarns.

5.2.7 *In vitro* bioactivity of dexamethasone release from multifilament yarns

Human aortic valve interstitial cells isolated from human cryopreserved conduits (hVIC, passage 2 through 4, a gift from the Cardiac Regenerative Surgery Research Laboratory, Children's Mercy Hospital) were used for *in vitro* studies. Cells were maintained in hVIC media, Dulbecco's Modified Eagle's Medium (DMEM F12, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco), 1% L-glutamine, 1% penicillin/streptomycin, and 1% amphotericin B. The biological activity of eluted DXM was determined using a cell proliferation assay similar to studies by Reil et al. [29]. hVICs (passage 5) were plated with 10% FBS growth media at 5000 cells/cm² into collagen type I coated 12-well plates (BD BioCoat) and cultured for 24 h. Cell growth arrested by washing plates with PBS and adding 1% FBS serum starvation media. Growth arrest was maintained for 48 h to allow for cell cycle synchronization. Synchronized cells were then re-stimulated with 10% FBS growth media containing 10⁻⁷ mol/L DXM. DXM-supplemented growth media was made using eluted DXM releasates from multifilament yarns at 1-day and 56-day incubation. Fresh, unencapsulated drug and PBS vehicle were used as controls. The media for each experimental group was changed every 24 h up to a total exposure of 72 h.

5.2.8 Mechanical testing

Uniaxial tensile tests were conducted using a materials testing system (Instron Model 4442) in accordance to the United States Pharmacopeia (USP) absorbable suture testing standards [30]. Monofilaments and yarns were secured to a paper frame (25 mm x 25 mm) and loaded into the crosshead clamps of the machine. Prior to loading, the sides of the paper template were cut leaving the sample intact. An elongation rate of 50 mm min⁻¹ was applied until failure and the load-displacement data was collected by the

digital acquisition system. Load at break is a measure of elongation until fiber fracture. The ductility, or strain at failure ($\varepsilon = \Delta L L_o^{-1}$) is calculated from the original gauge length (L_o) and the change in gauge length (ΔL) as recorded by crosshead movement until fiber fracture. Ten replicates of each formulation were analyzed.

5.2.9 Statistical analysis

Statistical analysis was performed using SPSS v.19 statistical software (Chicago, IL). All data are expressed as mean $\pm$ standard deviation (S.D.). A one-way analysis of variance (ANOVA) and *post hoc* Tukey multiple comparisons test was used for group comparisons. To determine the significance of theoretical multifilament yarn drug release in comparison to experimental release, a two-tailed *t*-test was performed. A *p* value of less than 0.05 was considered statistically significant.

5.3 Results

In wet spinning, an initially homogeneous polymer solution is extruded into a coagulation bath that induces phase inversion by counter-diffusion of solvent and nonsolvent (**Figure 5.1A**). Phase separation of the polymer solution is then initiated into two phases, a polymer lean and polymer rich phase. Phase separation of polymers takes place during the amount of time the fiber is left in the coagulation bath, referred to as the residence time. While wet spinning is a versatile technique for the production of continuous monofilament fibers, it is also the most complicated spinning method. The size, shape, morphology, and strength of wet spun filaments depend on different factors like polymer concentration, solubility of polymer in solvent, solvent/nonsolvent miscibility parameters, residence time, fiber drawing methods, etc. This paper breaks down the complexity of wet spinning into the processes contributing to mechanical

strength and drug delivery attributes, and begins to recombine these processes to develop multifunctional fiber platforms towards the design of drug-eluting sutures.

5.3.1 Morphology of wet spun monofilaments

Throughout our studies, we observed that we could fabricate fibers with diverse surface structure by applying post-handling techniques, varying spin dope precipitation strength, and altering the solvent/nonsolvent miscibility parameters. For example, as-spun PLGA fibers extruded into petroleum ether possessed a smooth surface morphology (**Figure 5.2A, B**). Conversely, solution-drawn PLLA_{0.94} fibers extruded into a mixture of 2-propanol and petroleum ether and stretched under tension as the dope solution precipitated displayed longitudinal grooves along the direction of fiber drawing (**Figure 5.2C, D**). Fibers spun from high and low solution concentrations and extruded under otherwise identical conditions also resulted in diverse surface architectures. Fibers wet spun from 10% (w/v) PLLA (**Figure 5.2F**) began gelation slower than 15% (w/v) PLLA (**Figure 5.2E**) fibers since there was more solvent volume to diffuse into the petroleum ether coagulation bath. Similarly, changing the molecular weight of the polymer solution altered the surface morphology wet spun filaments. High molecular weight PLLA (**Figure 5.2G**) solutions had smoother surface structures in comparison to lower molecular weight PLLA_{0.94} (**Figure 5.2H**) solutions spun under identical conditions. Altering the nonsolvent bath composition changed the surface roughness of wet spun filaments. Composite spin dope solutions extruded into petroleum ether displayed smooth surface topography (**Figure 5.2I**), whereas the same solution extruded into a 50:50 mixture of 2-propanol to petroleum ether possessed surfaces with micron-rough grooves and spherical protrusions (**Figure 5.2J**). Solutions precipitated visibly slower in 2-propanol than petroleum ether. The changes we observed in the rate of precipitation are a direct result of the solvent system that we used. The solubility parameter of DCM ($\delta_{\text{DCM}} = 20.2 \text{ MPa}^{1/2}$) is 5.5 units higher than petroleum ether (PE)

($\delta_{PE} = 14.8 \text{ MPa}^{1/2}$) and 3.6 units from 2-propanol (2-P) ($\delta_{2-P} = 23.8 \text{ MPa}^{1/2}$), making petroleum ether a better nonsolvent for the polymer. Thus, the onset of precipitation is faster with petroleum ether and a smooth surface morphology is obtained.

Composite formulations selected for multifilament yarn production further demonstrate the diverse effects of processing conditions on surface structure and cross-sectional morphology of wet spun filaments. Post-drawn 10% (w/v) composite fiber surfaces had longitudinal striations with many spherulites (**Figure 5.3A, C**) in comparison to 20% (w/v) composite fibers, which had nano-porous surfaces and fewer spherulites (**Figure 5.3E, G**). The cross-sectional porosity of composite fibers was also significantly different. The porosity of 10% and 20% (w/v) composite fibers was $3.0 \pm 1.8 \text{ } \mu\text{m}^2$ and $9.1 \pm 2.8 \text{ } \mu\text{m}^2$, respectively.

5.3.2 Drug release kinetics of as-spun solution-drawn monofilaments

To investigate the effect of polymer composition and concentration on DXM release, the kinetics of drug release from composite monofilaments of 10% and 20% (w/v) polymer concentrations were determined. Polymer concentrations were selected based on the mechanical characteristics of blank as-spun PLLA fibers (**Figure 5.4**). Initial release studies were carried out to 35 days *in vitro* to mimic the timeline of acute and chronic phases of wound healing. The cumulative DXM release from 10% (w/v) composite monofilaments loaded with 2.5% (w/w) DXM is shown in **Figure 5.5A**. Each formulation exhibited an initial burst release from the first day of incubation, followed by a continuous linear release up to 35 days. The initial release of drug was fastest from fibers prepared using more hydrophilic polymers. PLLA/PLGA/PVP composite fibers (SF-10b) had the highest initial burst of $5.5 \pm 0.7 \text{ } \mu\text{g mg}^{-1}$ of fibers, while all other formulations had similar initial drug burst release of $2.5 \pm 0.5 \text{ } \mu\text{g mg}^{-1}$ of fibers. In the presence of PLGA, PVP addition had a noticeable increase over initial burst. PLLA/PLGA/PVP (SF-10b) and PLLA/PLGA (SF-10a) released the greatest overall

drug release (50% and 60% encapsulated DXM) with average daily release rates of $1.1 \mu\text{g mg}^{-1}$ and $1.8 \mu\text{g mg}^{-1}$ of fibers, respectively. PLLA/PVP (SF-10e), PLLA/PLLA_{0.94}/PVP (SF-10d), and PLLA/PLLA_{0.94} (SF-10c) formulations released 24-29% total encapsulated drug and had similar daily release rates of $0.6\text{-}0.7 \mu\text{g mg}^{-1}$ of fibers.

The release kinetics of 20% (w/v) composite fibers loaded with 1.5% (w/w) actual DXM are shown in **Figure 5.5B**. Composite fibers were made from ratios of 1:1 (SF-20a), 1:2 (SF-20b), 1:3 (SF-20c), and 1:4 (SF-20d) PLGA to PLLA (20-50% PLGA content). Fibers prepared from 20% (w/v) spin dope solutions exhibited significantly different release kinetics in comparison to fibers wet spun from 10% (w/v) polymer solutions. All PLGA/PLLA formulations exhibited very little burst ($<3\%$ encapsulated DXM) within the first day of incubation, despite the addition of PLGA up to 50% fiber composition. Each formulation had a similar lag phase until 14-day incubation. At this phase, the formulation with 50% PLGA content showed a marked increase in release rate. After 35 days, the cumulative drug release was $13.6 \pm 2.7 \mu\text{g mg}^{-1}$ of fibers (78% encapsulated DXM) for 1:1, $2.3 \pm 0.2 \mu\text{g mg}^{-1}$ of fibers (21% encapsulated DXM) for 1:2, $1.7 \pm 0.7 \mu\text{g mg}^{-1}$ of fibers (12% encapsulated DXM) for 1:3, and $1.8 \pm 0.8 \mu\text{g mg}^{-1}$ of fibers (12% encapsulated DXM) for 1:4 PLGA/PLLA formulations. Drug release from 20% (w/v) polymer solutions was only significantly increased with the addition of 50% PLGA content.

5.3.3 Drug release kinetics of post-drawn composite monofilaments and ‘Z’ twisted multifilament yarns

The current work focuses on engineering drug-eluting wet spun microfilaments into macro-structured implants. Although the crystalline regions of PLLA contribute to mechanical strength, it can also decrease the rate of drug release. Previous studies by our group have shown the linear release of up to 28% total encapsulated DXM from

PLLA fibers after eight weeks *in vitro* [28]. For the previous study, it was possible to achieve therapeutic levels of DXM from wet spun fibers using a low (7.5%) PLLA concentration. To increase the rate of release of DXM from higher PLLA concentrations (10% and 20% w/v) we chose to make composite fibers with the addition of a lower molecular weight PLLA, amorphous PLGA, and water-soluble polymer PVP.

Monofilaments used for yarn fabrication were spun from 10% and 20% (w/v) concentrations. Polymer blends were selected to achieve therapeutic ranges of DXM treatment (**Table 5.1**). The PLGA and PVP content was increased for 20% (w/v) composite fibers to further modulate release. The release kinetics of post-drawn monofilaments are shown in **Figure 5.6A**. Monofilaments spun from 10% (w/v) composite fibers loaded with 2.8% actual DXM had substantially less burst release in comparison to 20% (w/v) polymer solutions loaded with 2.4% actual DXM.

After the initial burst phase, fibers made from 10% (w/v) fibers exhibited linear release rates ($R^2 = 0.98$) in comparison to 20% (w/v) solutions, which exhibited logarithmic drug release ($R^2 = 0.96$) up to 56 days *in vitro*. The average daily release after the initial burst for composite solutions was $0.7 \mu\text{g mg}^{-1}$ for 10% (w/v) fibers. For 20% (w/v) fibers, the average daily release up to 21-day incubation was $1.8 \mu\text{g mg}^{-1}$, followed by a release rate of $0.2 \mu\text{g mg}^{-1}$. At 56-day incubation, 10% (w/v) formulations released $11.6 \pm 0.4 \mu\text{g mg}^{-1}$ of fibers (21% encapsulated DXM), whereas 20% (w/v) formulations released $17.2 \pm 0.4 \mu\text{g mg}^{-1}$ of fibers (36% encapsulated DXM).

Post-drawn fibers could potentially be used to tailor the release of therapeutics from medical implants by the formation of multifilament yarns. Yarns produced from several different combinations of single fibers could achieve desired release profiles for specific clinical applications, which could include the sequential release of multiple small molecules or therapeutics. **Figure 5.6B** shows five different theoretical 6-ply yarn release profiles predicted from the experimental release of single fibers in **Figure 5.6A** using equation 1. To determine the fit of our model, the experimental DXM release

from a 6-ply multifilament yarn formulation was evaluated. The release kinetics of 6-ply multifilament yarns produced by ‘Z’ twisting four, single filaments of formulation ‘A’ with six, single filaments of formulation ‘B’ (**Table 5.1**) had release profiles within the ranges of individual filaments (**Figure 5.6C**). Yarns released $5.3 \mu\text{g mg}^{-1}$ (15% encapsulated DXM) initial burst release after the first day of incubation, followed by an average daily release of $0.8 \mu\text{g mg}^{-1}$ from day 1 to day 56 incubation. At 56-day incubation, yarns released a total of $12.4 \pm 1 \text{ mg}^{-1}$ (35% encapsulated DXM). Overall, there is a good agreement between the experimental drug release and the theoretical prediction. Release samples at all time points except days 14 and 28 were not statistically different ($p < 0.05$) than the predicted release, suggesting monofilament twisting did not affect the release kinetics of constituent monofilaments. Therefore, we can assert the linearity of our system.

5.3.4 Biological activity of eluted dexamethasone from multifilament yarns

The effect of dexamethasone released from multifilament yarns (section 3.2) on the proliferation of hVICs was studied to evaluate the biological activity of eluted drug. The proliferation of hVICs cultured in the presence of DXM-supplemented media from multifilament yarns that had been incubated for 1 day and 56 days in comparison to control media is shown in **Figure 5.6**. hVICs cultured in the presence of medium that contained DXM shows that the cells are proliferating at a significantly reduced rate after 72 hours exposure in comparison to control cells with PBS vehicle. As well, the proliferation of hVICs grown in medium that contained fresh and eluted DXM are similar. These findings confirm that wet spinning does not alter the biological activity of encapsulated DXM since both eluted and unencapsulated DXM treatment had the same effect on the growth and proliferation of hVICs.

5.3.5 Mechanical properties of post-drawn monofilaments and ‘Z’ twisted multifilament yarns

The tensile properties of wet spun fibers wound around metal bobbins were evaluated to study the effects of post-drawing and multifilament yarn production on filament breaking strength and ductility. A summary of the tensile properties of single filaments (SF) and twisted (TW) 6-ply yarns shown in **Table 5.2**. After fabrication, blank and drug-loaded monofilaments had a slight increase in load to failure after increasing the polymer concentration from 10% to 20% (w/v). Addition of DXM into wet spun monofilaments did not significantly reduce the breaking strength of fibers, unlike other studies encapsulating drugs within wet spun fibers [31-33]. The ductility of 10% and 20% (w/v) blank ($p = 0.061$) and drug-loaded ($p = 0.155$) were also similar. DXM-loaded and blank multifilament yarns possessed a fivefold increase ($p = 0.000$) in load bearing capacity in comparison to individual DXM-loaded and blank fibers. The marked increase in load at failure correlated well with the summation of the failure strength of individual monofilaments. The ductility of yarns was significantly reduced ($p < 0.05$) in comparison to individual blank and drug-loaded fiber formulations, indicating monofilament twisting removed some of the strain within constituent monofilaments.

5.3.6 Handling capabilities of ‘Z’ twisted multifilament yarns

To demonstrate the ability to scale up of microfilaments into complex structures, single microfilaments were grouped together and twisted into multifilament yarns. Six fibers were twisted in the ‘Z’ direction for the formation of 6-ply multifilament yarns. Twisted fibers could be scaled to a thickness of around 150 μm with even entwining and small spaces between single fibers (**Figure 5.1B**). The handling capabilities of multifilament yarns improved in comparison to constituent monofilaments. Yarns could easily be braided, knitted or woven into complex geometries (**Figure 5.8**).

5.4 Discussion

The objective of this research was to fabricate biologically active fibers that could be integrated into existing biomedical implants. A bench-top technique for the scale up of monofilaments into multifilament yarns was developed to enhance the handling capabilities of wet spun filaments and to demonstrate the ability to tune drug release kinetics. Local, controlled delivery of anti-inflammatory drugs to surrounding tissues could help reduce unwanted inflammatory cell infiltrates and increase the longevity of implanted biomaterials.

The wet spinning technique used in this study is a simple and versatile method for the production of continuous micron-sized fibers. By altering wet spinning parameters, we were able to manipulate the surface topography of wet spun filaments. In general, quick quenching yields fibers with smooth surfaces [34]. Therefore, it was possible to create fibers with micrometer-range features such as grooves, ridges, and spherical protrusions by decreasing the polymer solution concentration, slowing the counter-diffusion of solvent and nonsolvent, altering the residence time in the coagulation bath, or simply applying tension through solution- and post-drawing methods. Altering the surface topography of wet spun fibers could be beneficial for host tissue integration and wound healing by enhancing contact guidance and cellular attachment [35].

To demonstrate the ability to modulate drug release from wet spun filaments, we produced fibers with varying polymer compositions, including excipients such as PVP, and solution concentrations. Drug-loaded formulations were as-spun, solution-drawn or post-drawn, depending on the rate of precipitation and counter-diffusion of solvent and nonsolvent. Each of these processes were developed to determine whether wet spun fibers can be used to design hybrid devices, ones that perform a mechanical function and simultaneously delivery drugs. These methods show the complexity of designing a wet spun delivery system and the scale up of monofilaments into multifilament yarns.

Overall, DXM release of wet spun filaments of similar polymer concentration was dependent on the hydrophilicity of the composite formulation. For 10% (w/v) solution-drawn composite formulations, the highest drug release rates were achieved by adding PLGA and PVP. Since PVP is a water-soluble polymer, increased drug release can be attributed to pore formation from the solubilization of PVP in buffer solution. After the initial burst period, the release of drug from solution-drawn 10% (w/v) formulations was favored by the swelling capability of the fibers. As-spun formulations with 20% (w/v) solutions containing PLLA and PLGA also showed the release of DXM was dependent on the hydrophilicity of the filaments. A substantial difference in drug release kinetics of 20% (w/v) composite fibers was achieved only with the addition of 50% PLGA content. The ability to prolong the release of drug was also apparent by increasing the polymer concentration from 10% to 20% (w/v).

Post-drawn composite formulations selected for multifilament yarn production had increased DXM release with increased PLGA and PVP addition, despite differences in overall polymer concentration. Fibers spun from 10% (w/v) solutions had reduced DXM burst release in comparison to fibers spun from 20% (w/v) solutions. For these formulations, variations in DXM release kinetics can be attributed to processing conditions. The PVP content in 10% (w/v) formulations was less than 20% (w/v) formulations, which could have resulted in the decrease in the initial burst release of DXM. Nano-sized pores on the surfaces of 20% (w/v) composite solutions could also have modulated drug release by increasing buffer penetration. Surface pore formation can be explained by the rapid evaporation of solvent entrapped within the precipitating fiber during winding. Filaments spun from 10% (w/v) solutions also displayed a dense cross-sectional morphology. Cross-sectional pores are caused by the entrapment of solvent during spin dope solution precipitation. Composite fibers from 10% (w/v) solutions had less polymer concentration to block the counter-diffusion of solvent and nonsolvent, resulting in significantly less cross-sectional porosity in comparison to 20% (w/v) solutions. A reduction in the void size of 10% (w/v) fibers could have also been

an artifact of fiber drawing, as has been shown elsewhere [36]. While both 10% and 20% (w/v) composite fibers experienced similar forces during drawing, 10% (w/v) solutions had less resistance to deformation as judged by the grooved surface topography.

The therapeutic range of DXM delivery to treat post-operative inflammation from various biomaterials has been evaluated in a number of animal models [37-41]. Hickey et al. evaluated the potential of localized DXM delivery from PLGA microspheres to suppress inflammatory responses to cotton threads implanted subcutaneously in rats [39]. Their studies showed DXM treatment was most effective when delivered as a burst release followed by a slow release ($3\text{-}30\text{ }\mu\text{g day}^{-1}$) over the course of 30 days *in vivo*. Other studies have evaluated microspheres and hydrogel composites for the continuous release of DXM to treat biomaterial-driven inflammation and found release rates of $0.17\text{-}7.2\text{ }\mu\text{g day}^{-1}$ were effective in modulating host immune responses [37, 40]. Researchers have also recently investigated the real-time inflammatory response to biomaterial implants using non-invasive fluorescence imaging techniques [38, 41]. These studies showed local delivery of DXM from PLGA microspheres succeeded to significantly reduce biomaterial-driven inflammatory responses up one month *in vivo*. Given these findings, it would be simple to combine formulations from this study to achieve DXM release rates well within the therapeutic ranges of drug treatment.

To engineer fibers with various release profiles of biologically active drug, post-drawn filaments were scaled up into 6-ply multifilament yarns. Yarn production did not affect the predicted release of DXM from individual filaments (**Figure 5.6C**). The biological activity of eluted drug was also preserved up to 56-day incubation. Drug-eluting yarns from this study could be formed from a combination of several types of individual monofilaments for the prolonged release of a multitude of therapeutics with retained biological activity. Drug release profiles could be further tuned to meet specific clinical needs by altering the polymer composition, molecular weight and concentration as

previously discussed. Additionally, wet spun fibers could be used as conduits for the long-term delivery of other biologically active drugs and/or proteins.

The tensile properties of post-drawn monofilaments and ‘Z’ twisted multifilament yarns were characterized to evaluate their use as multifunctional delivery systems. Overall, increasing the polymer concentration did not result in increased tensile properties as was found with blank fibers spun from PLLA only (**Figure 5.4**). It has been established that polymer chains align in the direction of shear flow during spinning [42]. Dilute polymer solutions have less polymer chains and more mobility to align in the direction of flow, promoting a higher degree of chain orientation along the fiber axis. Thus, it is possible that the molecular orientation and crystalline morphology of post-drawn 10% and 20% (w/v) fibers are different. Post-drawing has been also been shown to increase the degree of orientation, density and fiber crystallinity [36, 43]. Longitudinal striations on the surfaces of 10% (w/v) fibers imply post-handling processes could have also influenced molecular orientation during fiber drawing. When fibers are under tension, such as in filament drawing, the amorphous units of the polymer begin to unfold, allowing for a reduction in free volume and amorphous polymer chain alignment until fracture.

Monofilaments spun from 10% (w/v) solutions had similar ductility as compared to 20% (w/v) solutions. One would expect increased polymer concentration to lead to increased strain at failure (**Figure 5.4**). However, the cross-sectional morphology of composite fibers was very different, unlike the as-spun fibers made from PLLA only (data not shown). The residence time of post-drawn composite fibers prepared for multifilament yarn fabrication was ~6 min, whereas as-spun PLLA fibers remained in the coagulation bath until all of the spin dope was extruded, yielding a residence time of ~1.5 hrs. Reduced residence time in the coagulation bath can lead to more porous fibers by decreasing the growth rate of voids and available time for growth during phase separation. Most importantly, drug encapsulation did not considerably affect the material properties of monofilaments.

The United States Pharmacopeia (USP) determines the standards specifying test procedures and product specifications for surgical sutures. Bioresorbable sutures must meet knot-pull tensile strength for approval by the USP. The average maximum tensile stress for DXM-loaded multifilament yarns was approximately 450 mN, or 0.05 kgf, equivalent in strength to a 9-0 (0.030-0.039 mm diameter) absorbable synthetic suture [30]. Although multifilament yarns are much larger in diameter than 9-0 sutures, their handling capabilities demonstrate the potential to hybridize wet spun fibers with existing surgical sutures to meet tensile strength specifications. Using textile industry embroidery technologies, it could be possible to weave, braid, or knit drug-eluting yarns with or around existing sutures. Since there was much strain left in the fibers after the wet spinning process, it is also possible to improve the mechanical properties of drug-eluting filaments through industrial-scale post-drawing techniques. Multifilament drug-eluting yarns from this study stretched over 100% of their initial length before failure. Mechanical stretching could be used to decrease the ductility of yarns and increase tensile strength. Stretching could similarly be applied to individual fibers prior to multifilament yarn formation.

5.5 Conclusions

We have developed a multifilament yarn with tunable DXM release kinetics that can be controlled through the combination of constituent monofilaments. The spatiotemporal release of therapeutics could be further controlled by the location of specific yarns within a 3-dimensional biomedical implant. We have also shown that the encapsulation of dexamethasone within wet spun fibers does not weaken mechanical strength or lead to fiber embrittlement. Although drug-eluting yarns were not as strong as conventional melt spun sutures, they were capable of physical manipulation and have the potential to be incorporated into existing biomedical textiles. This work touches upon the possibility of making micron-scale alterations to the surface topography of wet spun fibers by

applying stretch and varying wet spinning processing conditions. The potential to alter the surface topography of monofilaments, tune the release kinetics of a biologically active therapeutics, and enhance the load bearing strength of wet spun fibers through multifilament twisting, makes wet spun yarns extremely valuable for many biomedical and drug delivery applications (**Figure 5.9**). The diverse release kinetics achieved in this study may be used for future *in vivo* examination to determine the ideal release profile of potential therapeutics to decrease non-specific inflammatory responses to implanted biomaterials.

5.6 References

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5.7 Tables

Table 5.1: Wet spun fiber formulations with DXM loading

	Polymer composition (% w/w)			
	PLLA	PLGA	PLLA0.94	PVP
Solution-drawn 10% solution				
SF-10a	95	5	—	—
SF-10b	94	5	—	1
SF-10c	95	—	5	—
SF-10d	94	—	5	1
SF-10e	99	—	—	1
As-spun 20% solution				
SF-20a	50	50	—	—
SF-20b	67	33	—	—
SF-20c	75	25	—	—
SF-20d	80	20	—	—
Post-drawn 10% solution				
SF-10, formulation ‘A’	94	5	—	1
Post-drawn 20% solution				
SF-20, formulation ‘B’	80	10	—	10

Table 5.2: Summary of the tensile properties of post-drawn monofilaments and ‘Z’ twisted multifilament yarns. Data are represented as mean $\pm$ S.D. ($n = 10$).

Sample type	Diameter (μm)	Load at failure (mN)	Strain at failure (mm/mm)
SF-10 _{blank}	49.5 ± 7.9	64.3 ± 7.7	2.10 ± 0.59
SF-20 _{blank}	70.2 ± 7.9	89.9 ± 8.7	1.48 ± 0.72
TW _{blank}	152.1 ± 6.3	341.5 ± 27.1	1.34 ± 0.35
SF-10	48.4 ± 6.6	71.0 ± 8.7	2.55 ± 0.51
SF-20	64.6 ± 6.9	77.3 ± 3.6	2.12 ± 0.59
TW	153.9 ± 7.5	447.1 ± 40.9	1.16 ± 0.40

SF, single fiber

TW, twisted yarn

blank, drug-free

5.8 Figures

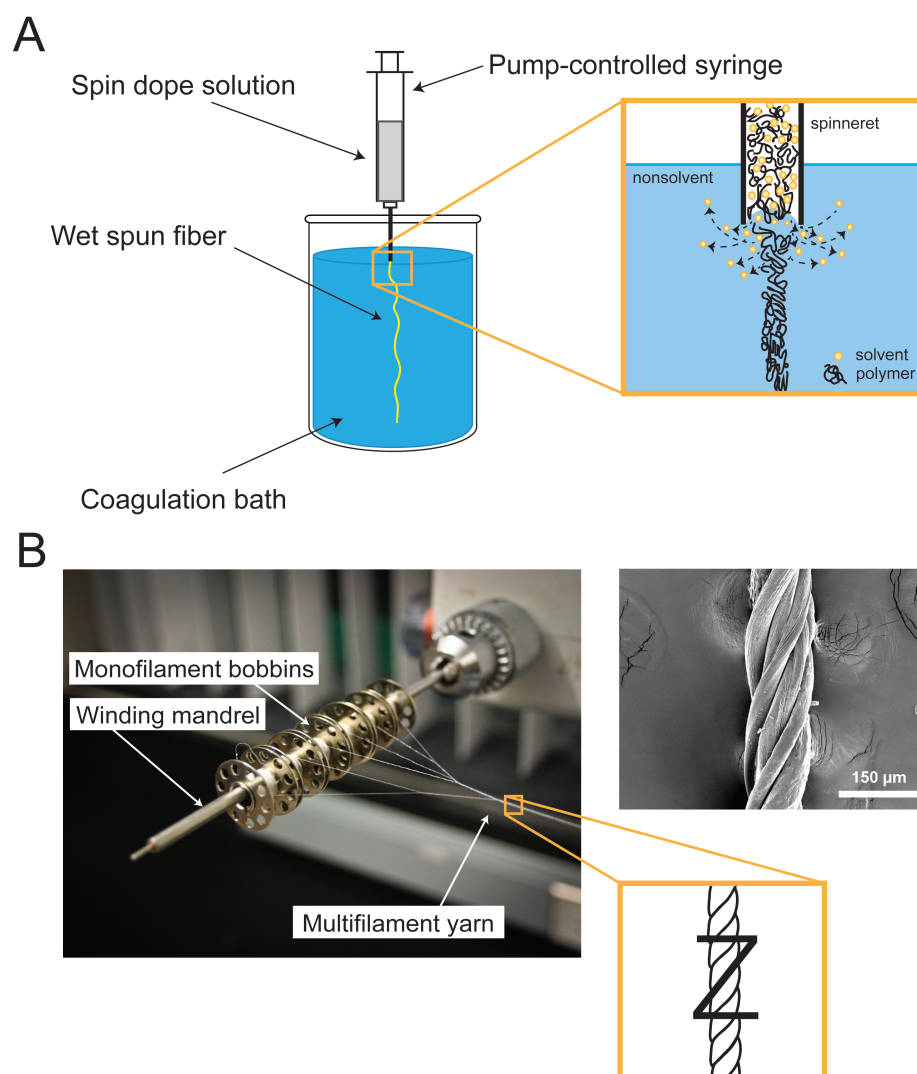


Figure 5.1: Details of wet spinning system and yarn formation, showing schematic representation of (A) the spinning apparatus and photographic representation of (B) the apparatus for multifilament yarn production.

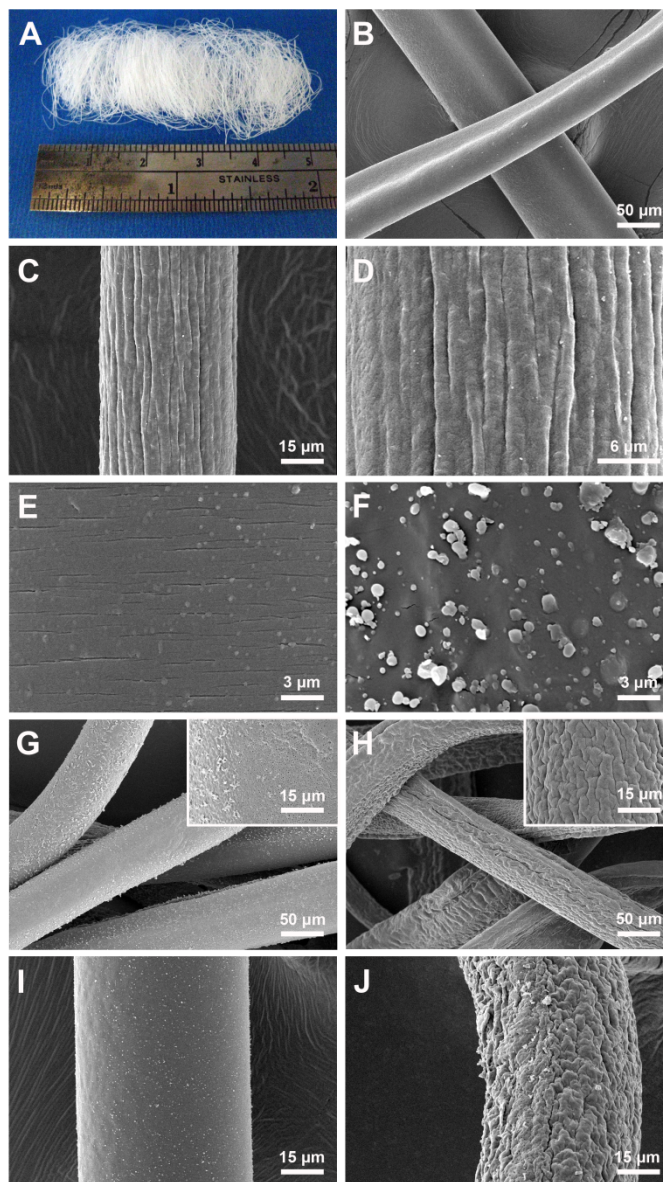


Figure 5.2: Fabrication of wet spun monofilaments with diverse surface structure. (A) Photographic image of as-spun 3D fiber bundle, 20% (w/v) PLGA extruded into petroleum ether. (B) Scanning electron micrograph (SEM) of fibers in panel A. (C, D) Post-drawn 7.5% (w/w) PLLA_{0.94} fiber extruded into a 75:25 ratio of 2-propanol to petroleum ether. (E) SEM of 15% (w/v) and (F) 10% (w/v) PLLA fibers extruded into petroleum ether coagulation baths. (G) 7.5% (w/v) PLLA fibers extruded into 50:50 ratio of petroleum ether to 2-propanol. (H) Wet spun 7.5% (w/v) PLLA_{0.94} fibers extruded under similar conditions as panel G. PLLA/PLGA composite fiber extruded into (I) petroleum ether, and (J) 50:50 ratio of petroleum ether to 2-propanol.

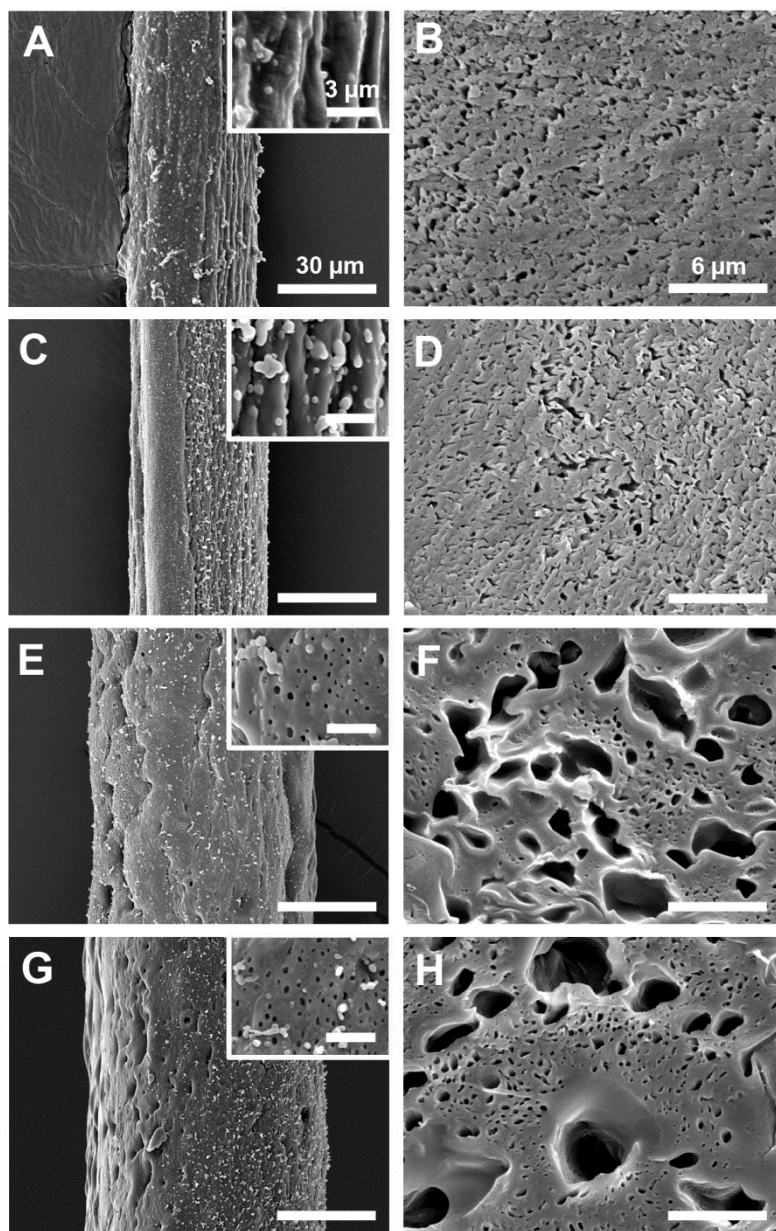


Figure 5.3: Post-drawn composite fibers have different surface and cross-sectional morphology. Scanning electron micrographs of (A, B, E, F) blank and (C, D, G, H) DXM-loaded fibers spun from (A-D) 10% and (E-F) 20% (w/v) spin dope solutions.

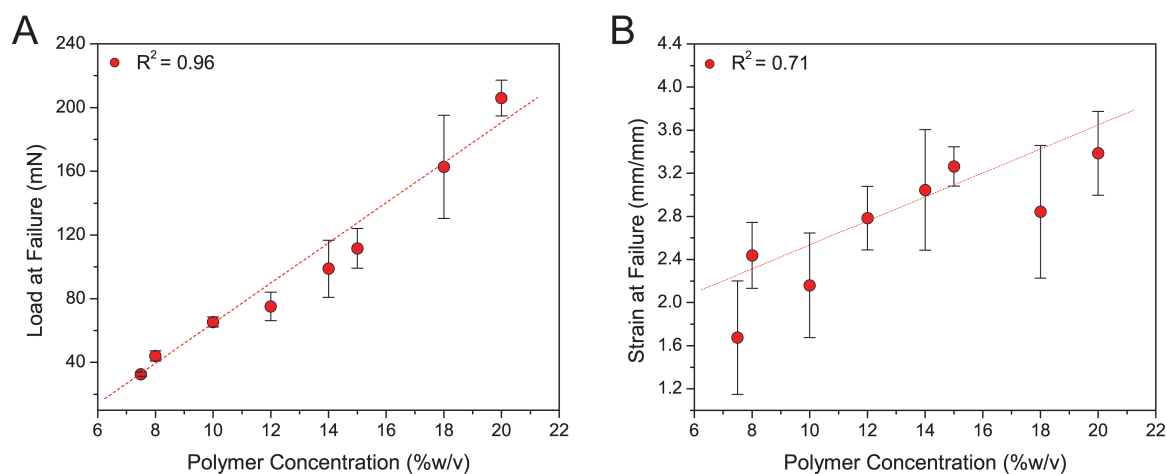


Figure 5.4: Polymer concentration affects the tensile properties of wet spun fibers. (A) Load at failure and (B) strain at failure for as-spun PLLA fibers extruded into petroleum ether.

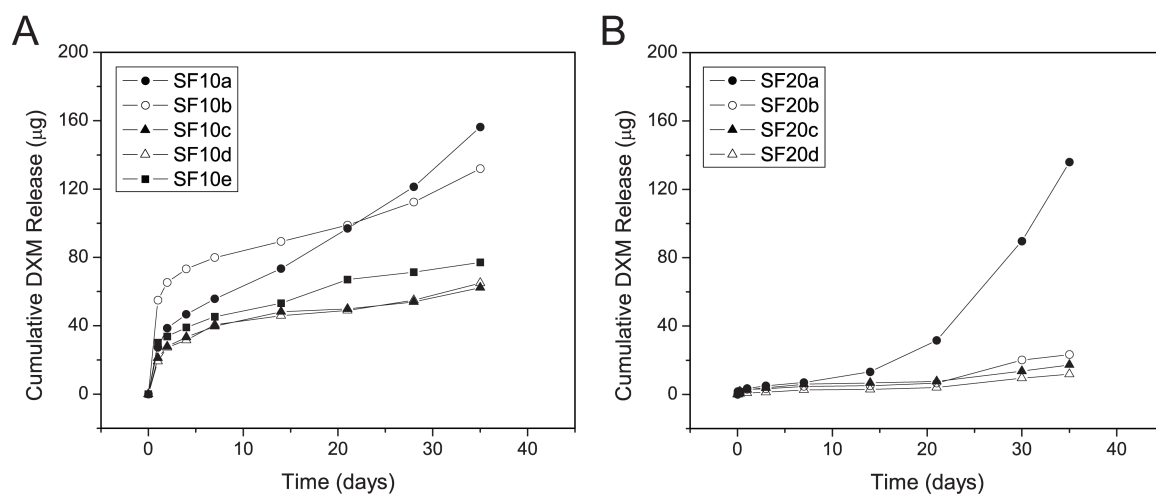


Figure 5.5: Polymer composition modulates drug release kinetics. Drug release profiles of 10 mg wet spun composite monofilaments prepared from (A) 10% and (B) 20% (w/v) polymer solutions loaded with 2.6 and 1.5% (w/w) DXM, respectively.

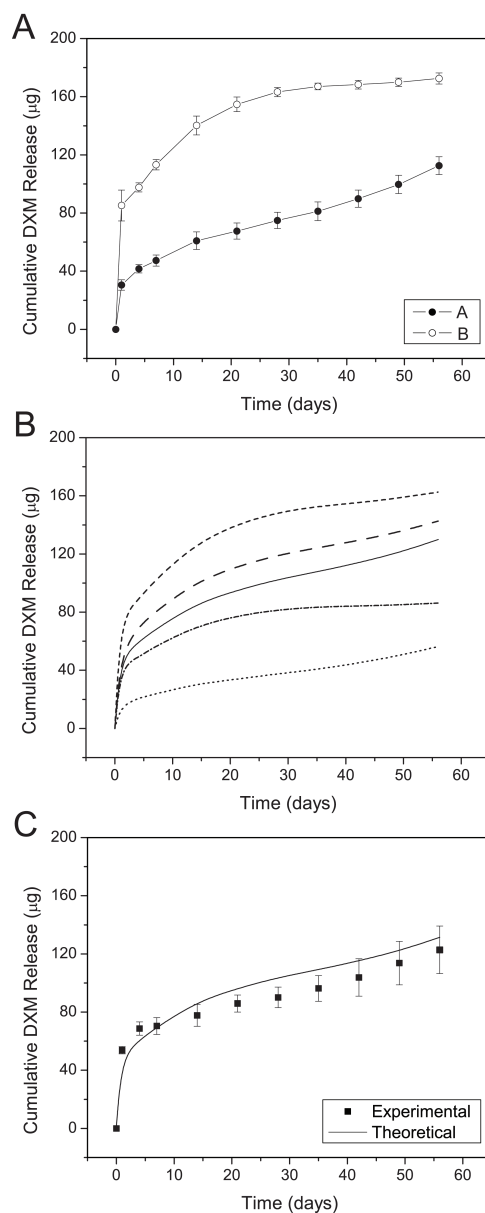


Figure 5.6: Multifilament yarns can be used to tune release kinetics. (A) Drug release profiles of 10 mg wet spun composite monofilaments prepared from 10% (black circles) and 20% (open circles) (w/v) polymer solutions loaded with 2.8 and 2.4% (w/w) DXM, respectively. Mean $\pm$ S.D. are presented. (B) Multiple theoretical predictions of the resulting drug release kinetics from DXM-loaded yarns as a function of single monofilament composition (6-ply yarn combinations of formulations 'A' and 'B' from the top to the bottom curve: 1A + 5B, 5A + 1B, 4A + 2B, 3A_{blank} + 3B, 3A + 3B_{blank}). (C) Drug release profiles of 10 mg 6-ply multifilament yarns produced by 'Z' twisting 4 monofilaments of formulation 'A' and 2 monofilaments of formulation 'B' (black squares) in comparison to the predicted release calculated using equation 5.1 (black line).

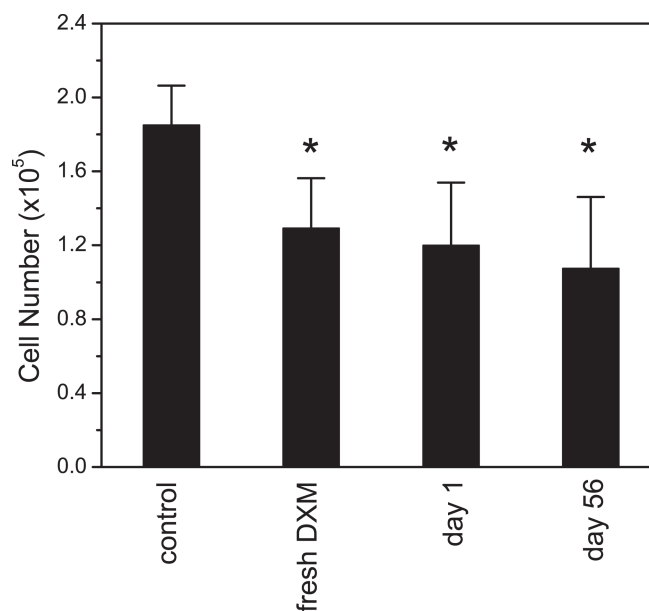


Figure 5.7: Dexamethasone treatment inhibits human aortic valve interstitial cell proliferation. Cell number was determined after 72 h exposure to 10^{-7} mol L⁻¹ DXM. DXM eluted from 6-ply multifilament yarns after 1 day and 56 days had the same biological activity as fresh, unencapsulated drug. Mean $\pm$ S.D. are presented. * $p < 0.05$ compared to control by ANOVA.

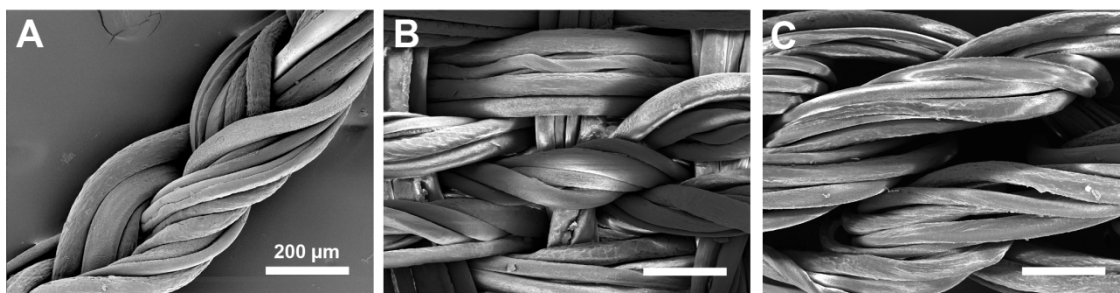


Figure 5.8: Handling properties of wet spun monofilaments are improved after multifilament yarn production. Scanning electron micrographs of (A) multifilament yarns (B) braided, (C) woven and (D) knitted into complex geometries.

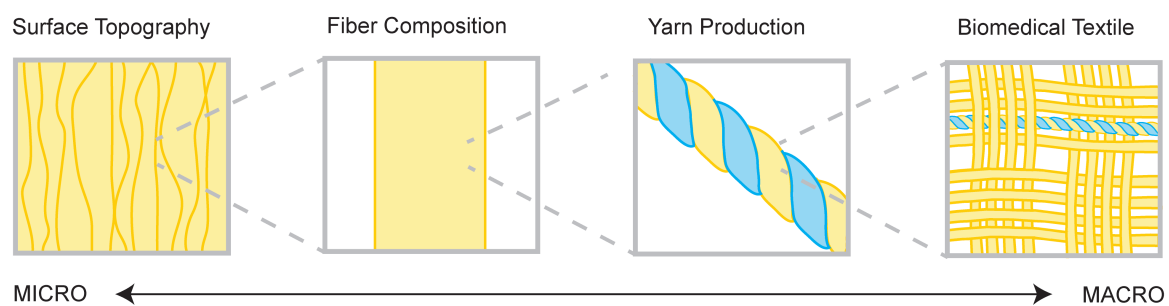


Figure 5.9: Cartoon representation of the four different levels of hierarchy in the design of therapeutic biomedical textiles. ‘Bottom-up’ approach demonstrates how wet spun filaments emerge as simple building blocks for the formation of macro-level scaffolds.

Chapter 6

Biocompatibility study of drug delivery sutures in an *in vivo* ovine model for cardiovascular tissue engineering applications

Cardiovascular research has focused on the development of tissue engineered valves, arteries, myocardial wall replacements, and other biological equivalents, all of which require surgical implantation. Fibrocalcifications present along the suture lines of such implanted tissues constrict the growth and functionality of the tissue *in vivo*. This is particularly critical in cardiovascular anastomoses in young children where growth of a tissue engineered construct is desired. If the suture lines do not grow, then the limitation will be at the surgical sites of implantation within the bloodstream. Thus, the need for new surgical interventions specifically applicable to the surgical implantation of tissue, cell and gene engineered constructs is critical. We have developed drug delivery sutures by braiding wet spun polymeric yarns containing dexamethasone and noggin around existing polypropylene sutures. The biocompatibility and therapeutic merits of encapsulated therapeutics were evaluated in a time-honored *in vivo* model for fibrosis

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and calcification. Drug delivery sutures positioned between the anastomoses of allograft valve conduits implanted in the right ventricular outflow tract of juvenile sheep showed significantly reduced inflammatory responses. These new drug-eluting sutures with suitable anti-inflammatory features appear to be a promising material for clinical and functional tissue engineering applications.

6.1 Introduction

Over 35,000 infants are born with a congenital heart defect each year in the United States. Nearly twice as many children die from heart defects than all forms of childhood cancer combined [1]. Approximately five percent of children with congenital heart defects require an aortic valve replacement [2]. The replacement of aortic valves in pediatric patients is viewed as a last resort due to unsatisfactory performance of valve substitutes. Mechanical valves are susceptible to several complications, including hemolysis, thromboembolism and infection. Porcine and pericardial bioprostheses develop rapid calcifications in children, resulting in the need for repeat operations [3, 4]. These responses are amplified in pediatric patients because of their ability to calcify tissue more rapidly than adults. Furthermore, mechanical and bioprosthetic valve substitutes do not allow for the recellularization and tissue in-growth necessary for the longevity of the implant in pediatric patients.

Difficulties with mechanical and bioprosthetic valves in the treatment of children with aortic valve disease have fueled interest in the development of biological human valve substitutes. Decellularized allograft tissues have been investigated as valvular substitutes because of superior structural and anti-thrombogenic properties. Allograft valves also possess the potential to recellularize *in vivo*, thus eliminating the need for multiple surgical interventions. Consequently, researchers have developed and patented methods for decellularizing/ recellularizing cryopreserved allograft tissues [4-7]. However,

decellularized pulmonary allograft tissues have been shown to develop calcifications localized to suture lines [7]. The long-term success of pediatric allograft valves requires that there be no calcifications in the surrounding tissue. Calcifications present along areas of suture injury constrict tissue growth and the ability of the valve to remain functional during somatic growth [5]. Therefore, the development of a suture drug delivery system could be used to prevent inflammation-stimulated fibrocalcification formation, thus improving the quality of life for many children.

Our interest is in developing drug-eluting sutures that can be used as a local therapeutic intervention to prevent fibrosis and tissue mineralization of allografts implanted in pediatric patients. Vascular fibrosis and tissue mineralization is thought to be a combination of mechanical and inflammatory stimuli. Bone morphogenic proteins (BMPs) are crucial mediators of inflammation-driven vascular calcification [8]. The expression of BMP-2/4 in human calcified plaque and calcifying vascular cells has also been observed *in vivo* [9, 10]. Vascular smooth muscle cells (VSMCs) in the presence of BMP-2/4 (produced from endothelial cells in response to injury) differentiate into osteoblast-like phenotypes. For these reasons, dexamethasone, a potent anti-inflammatory drug, and noggin, a cystine knot-containing BMP antagonist that blocks the BMP signaling pathway, were selected as potential therapeutics for the current study.

In the previous chapters of this thesis, we have carried out the development of drug delivery yarns by twisting single wet spun filaments, capable of encapsulating a wide variety of therapeutics, to achieve multifilament bundles. In the present study, we aimed to develop yarns encapsulating dexamethasone and noggin, and to integrate these yarns with existing polypropylene sutures to evaluate their biocompatibility and therapeutic potential *in vivo*. The juvenile ovine animal model was selected for this study because it is a time-honored model for vascular healing and suture-line calcification. As well, our extensive surgical experience with this particular surgical model was practical for the implantation of drug-eluting yarns.

6.2 Materials and methods

6.2.1 Spin dope preparation

Four formulations of polymeric monofilaments were made using two distinct spin dope preparation methods: dexamethasone-loaded (DXM) fibers were fabricated using Method I, and noggin-loaded (NOG) were made using Method II. These formulations are listed in **Table 6.1**. In Method I, DXM formulations were made by dissolving polymer mixtures in 5 mL a co-solvent mixture of dichloromethane (DCM, Fisher) to tetrahydrofuran (THF, Fisher) at 6:1 (v/v) for 24 h. DXM was then added directly to the polymer/solvent solution and mixed for 3 h prior to spinning. In Method II, NOG formulations were made using a two-step stabilization and micronization process. In the first step, 25 mg of Tween 20 (Sigma), Tween 80 (Sigma), PEG 4500 (Spectrum Chemicals), and Pluronic F-127 (Spectrum Chemicals) were weighted in separate 1 mL cryovials and 1 mL of HPLC water was added to each vial. Next, roughly 50 mg of sucrose (Spectrum Chemicals), glycine (Spectrum Chemicals), and PVP (Spectrum Chemicals) were weighed in separate 1 mL cryovials; similar to before, 1 mL of HPLC water was added to each vial. Each of these excipients was filtered using a 0.2 μm filter. Approximately 4 mL of 99% tert-butyl alcohol was added to four glass vials and 6 μL of the solutions containing Tween 20, Tween 80, PEG 4500 and Pluronic F-127 were added to each vial. In addition, 57 μL of the solutions containing sucrose, glycine, and PVP were added to each vial. Next, 400 μL of HPCL water containing 50 μg of NOG was added to each vial. Vials were then placed in a shelf lyophilizer and dried for 36 h, yielding a polymer cake. Prior to wet spinning 1.125 mL or 2.0 mL of polymer/solvent solutions were added to the dried polymer cake and sonicated for 30 s.

6.2.2 Monofilament fiber spinning

For the formation of continuous polymer monofilaments, spin dope solutions were added into a syringe fitted with a 22-gauge blunt tipped syringe needle and loaded into a syringe pump. Spin dope solutions were extruded into a nonsolvent coagulation bath for 1.5 m at 0.04–0.08 mL min⁻¹. DXM formulations were extruded into a 2 L 1:1 (v/v) mixture of 2-propanol to petroleum ether, while NOG formulations were extruded into 2 L of petroleum ether only. For the formation of NOG-loaded PLGA formations, 2% (v/v) Span-85 was necessary to prevent coagulation around the spinneret tip. After formulations were extruded, they were allowed to cure in the nonsolvent bath for 4 m, and then moved to an empty beaker and wound around metal bobbins in preparation for multifilament yarn formation.

6.2.3 Multifilament yarn formation and suture braiding

Multifilament polymeric yarns were fabricated similar to the method discussed in Chapter 5. Six (DXM) or four (NOG) monofilament fibers were twisted along the longitudinal axis in the ‘Z’ direction (filament inclination from top right to bottom left). The resulting 6-ply DEX multifilament yarn and 4-ply NOG multifilament yarn (one each) was then braided in a figure-eight pattern around a 6-0 prolene suture using an automatic braider. To prevent unraveling, two-part epoxy was used at the end of each 30 inch braided suture, weighted with a fishing sinker, and allowed to dry overnight. The sinker was then cut from the bottom of the drug delivery and attached to french eye suture needles. Sutures were wound around a sterile metal bobbin. The ends of sutures were trimmed during implantation to ensure none of the epoxy was left behind.

6.2.4 Experimental groups

A total of ten valve conduits were evaluated in this study, which included five cryopreserved pulmonary valves and five decellularized pulmonary valves. Pulmonary valves were harvested from male domestic crossbred sheep and either preserved using clinically analogous protocols or decellularized according to previously published methods [11]. Cryopreserved ($n = 3$) and decellularized ($n = 3$) valves were implanted with drug delivery sutures and compared to cryopreserved ($n = 2$) and decellularized ($n = 2$) valves implanted with prolene sutures as a control. All valves were bicuspidized prior to implantation with the exception of one cryopreserved and one decellularized valve in the drug treatment group.

6.2.5 Surgical procedure

Drug-eluting sutures were evaluated in implanted valve conduits in pre-esterus juvenile female crossbred domestic sheep (North Country Chevoit cross) approximately 30 to 40 kg. All procedures and post-operative care were compliant with NIH guidelines (National Institutes of Health publication No. 85-23) and were approved in advance by the Institutional Animal Care and Use Committee. Prior to surgery, animals were anesthetized (propofol, 4-6 mg/kg) and intubated, followed by administration of isoflurane anesthesia (1.5%-2.5%). Valve conduits were implanted aseptically with cardiopulmonary bypass support in the right ventricular outflow tract as interposition grafts in juvenile sheep after excising the native pulmonary valve cusps and using standard surgical techniques. Although drug delivery suture prototypes could easily be pulled through cryopreserved valves prior to shipment to our animal facility, the freeze-thaw process significantly altered handling capabilities. Polymer yarns became more brittle with shipment (dry ice) and storage conditions (-80°C), which ultimately led to fracture and fraying upon implantation. Consequently, only one load-bearing prototype was implanted in the distal anastomosis of an implanted valve conduit. Thus, for the

remainder of our experiments, drug delivery sutures were placed between the distal and vertical (bi-cuspidized conduits) anastomoses of re-approximated tissues. It is important to note that we did notice polymer yarns that were shipped on blue ice and kept at -20°C seemed to maintain their handling properties and this shipment and storage protocol should be used in future animal studies.

6.2.6 Echocardiography

Transesophageal echocardiography (TEE) was used to evaluate the conduit hemodynamic performance and assess microcalcifications at 20 post-implantation. Two-dimensional images and Doppler-derived hemodynamic measurements of the conduits were digitally captured (X7-2T probe; iE33 Phillips). Doppler-derived blood flow velocities were used to determine cardiac output (CO). The modified Bernoulli equation ($\Delta P = 4V^2$) was used to calculate mean and peak pressure gradients.

6.2.7 Implant retrieval and histological preparations

All animals were necropsied and tissue samples of spleen, kidney, liver, lungs, and ventricles were placed in 10% neutral buffered formalin. Explanted conduits were photographed (Sony CyberShot) and underwent gross, macroscopic (SteRE Discovery V12, Zeiss), and radiographic (Faxitron-LR, Raxitron X-Ray LLC) examination. Explanted suture anastomoses were fixed in Histo-Choice-MB (Amersco Inc) prior to paraffin or resin-embedding. Longitudinally sectioned distal and vertical anastomoses were used for hematoxylin and eosin (H&E) staining (American Histo Laboratory).

6.2.8 Microscopy studies

An Aperio ScanScope (Axiovision Technologies) was used for viewing microscopic images and analyzing morphometric characteristics of H&E stained tissue sections. The intensity of inflammation at the site of drug delivery sutures and control sutures was estimated by measuring the cellular composition around the suture voids of sectioned anastomoses. The numbers of cells were counted using Aperio ImageScope software and normalized to the selected areas of tissue.

6.2.9 Inductively coupled plasma optical emission spectroscopy (ICP-OES)

ICP-OES was used to quantify the amount of ionic concentrations of calcium (Ca) and phosphorous (P) in sutured anastomoses tissue specimens. Briefly, lyophilized tissue samples were acid hydrolyzed with 6 *N* HCl at 80°C for 4 h. Residual solvent was then evaporated with nitrogen gas and heating at 70°C. Samples were reconstituted with 1 mL 0.01 *N* HCl and diluted 1:25 in ICP diluent before analysis. Ca and P were determined using an ICP-OES Optima 7000 DV (Perkin Elmer) using appropriate standards of known concentration. Ca and P are expressed as mg g⁻¹ of the dry mass of tissue.

6.2.10 Real-time RT-PCR

Total RNA from tissue specimens was isolated with an RNAeasy Micro Kit (Qiagen) and converted into double stranded cDNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). cDNA was quantified with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). Real-time PCR analysis was performed (500 ng per reaction) using custom ovine TaqMan primers and probes on an

Applied Biosystems 7300 Real-time PCR System (Applied Biosystems). Quantitative levels for all genes were normalized to endogenous GAPDH. Subsequent fold change calculations were determined based on thermocycler generated relative quantitation data using the comparative $2^{-\Delta\Delta C_t}$ method. Primer sequences are available upon request.

6.2.11 Statistical analysis

Statistical analysis was performed using SPSS v.19 statistical software. The Student's t-test was used for comparisons between treatment groups. An $\alpha = 0.05$ level was used to determine statistical significance.

6.3 Results

6.3.1 Histological study of sutures

From the *in vitro* results, the drug eluting yarns were loaded with dexamethasone and noggin (**Table 6.1**) and studied for tissue response. During the surgical procedure, drug delivery yarns began to unravel due to the rigors of valve implantation and handling. Thus, yarns were positioned at the interface of re-approximated tissues between the distal and vertical suture anastomoses. After 20 weeks implantation, microscopic examination of the postoperative sutures showed drug-eluting yarns were still intact (**Figure 6.1**). None of the animals in this study exhibited rejection of the yarns, suture disjunction, or any other adverse reaction. The tissue reactions to treatment groups are shown in the optical micrographs of histological sections at week 20 (**Figure 6.2**). H&E stained anastomoses revealed differences in the tissue response to the treatment groups. Drug delivery yarns hybridized to polypropylene sutures led to a marked reduction in inflammatory cell infiltrates surrounding implanted sutures compared to the untreated group. To quantify the immune responses to surgical sutures with and without

treatment, the number of cells surrounding implanted sutures were counted and normalized to total area. As shown in **Figure 6.3**, anastomoses with drug delivery yarns contained tissue with significantly lower ($p < 0.01$) cell density in comparison to untreated controls. These histological studies exhibit favorable tissue response to drug eluting yarns indicating biocompatibility.

6.3.2 ICP-OES studies

The amount of calcium and phosphorous among the arms of this study was quite variable. For these reasons, implanted valves were grouped together by treatment, regardless of conduit (cryopreserved, decellularized) or anastomosis (distal, vertical) type. The results of ICP-OES studies are shown in **Figure 6.4**. The gross, macroscopic, and radiographic appearance of explanted conduits with and without treatment were similar. Dense, mineralized tissue was observed along the suture lines of all specimens. The amount of calcium present in anastomoses with and without drug treatment was not significantly different ($p = 0.051$). However, less phosphorous was detected in untreated anastomoses ($p = 0.030$) in comparison to groups with drug treatment.

6.3.3 TEE evaluation

The hemodynamic performance of implanted valve conduits accessed using transesophageal echocardiography (**Table 6.2**). No statistical differences were found between different treatment types for mean gradient ($p = 0.64$), peak gradient ($p = 0.97$), or cardiac output ($p = 0.36$). As well, no differences were observed among implanted annulus diameters of treatment groups ($p = 0.84$). The following homograft conduit wall calcification scores were noted: none (treated, $n = 1$; untreated, $n = 1$), spotty (treated, $n = 4$; untreated, $n = 2$) and extensive (treated, $n = 1$; untreated, $n = 1$).

6.3.4 Suture anastomoses gene expression

Twelve genes primarily involved in inflammation, wound healing and osteogenesis were selected for pilot RT-PCR studies. As shown in **Table 6.3**, genes were differentially expressed among treatment groups. Nearly all genes were down-regulated with drug treatment, with the exception of smooth muscle actin, bone morphogenic protein-4 and decorin. However, since the relative changes in gene expression between treated and untreated groups were low (< 1.5), further RT-PCR studies were not conducted.

6.4 Discussion

The objective of this research was to hybridize drug delivery yarns with existing surgical sutures and to evaluate their biocompatibility *in vivo*. A braiding technique for the integration of yarns with 6-0 ($\sim 70 \mu\text{m}$) polypropylene sutures was developed to create drug delivery sutures that could be surgically implanted. Local delivery of therapeutics at the interface of implanted biomaterials could help reduce host inflammatory response and improve long-term performance.

Host tissue integration has been identified as an important design attribute for many functional tissue engineering applications [12-14]. Autologous recellularization is especially critical for implanted decellularized tissues [7, 11, 15, 16]. *In vivo*, implanted valve conduits of the same type (cryopreserved or decellularized) with and without drug treatment showed similar cellular infiltration, with the exception of the tissues directly surrounding implanted sutures. Anastomoses with drug treatment had significantly reduced inflammatory responses to implanted sutures. The drug treatment associated with reduced inflammatory responses remains unclear, but it is likely related to the local delivery of dexamethasone. Dexamethasone has been shown to inhibit local inflammation in a variety of experimental models [17-21] and clinically approved pacemaker leads and cardiovascular stents [22-24]. It has also been shown that

dexamethasone may be useful in the treatment of pathological conditions in which chronic inflammation is involved, such as ophthalmological diseases [17, 25]. The results of this biocompatibility study are encouraging and invaluable for the design of future drug delivery suture prototypes.

This work also touched upon the possibility of valve bicuspidization as a surgical solution for mismatched allografts. The hemodynamic performance of bicuspidized valve conduits from this study is consistent with previous research with tricuspid valve conduits [11]. These results are encouraging and help to validate the use of the bicuspidized pulmonary valve model in futures studies.

The potential for drug eluting yarns to suppress tissue mineralization was evaluated by ICP-OES, radiography, TEE, and RT-PCR. Calcific responses were not substantially different with the therapeutics tested, suggesting that these molecules do not play a large role in suture-driven tissue mineralization. These results necessitate the evaluation of other soluble factors to block or enhance molecules that have been indicated wound healing and scarring and tissue mineralization. For example, inflammation-triggered expression of osteopontin (OPN) has been shown to hinder the rate of wound repair and contribute to fibrosis and scarring [26]. Thus, local delivery of therapeutics to block OPN expression may constitute a clinically relevant therapeutic intervention. As well, adrenomedullin has been shown to enhance wound healing in a number of tissues and may be of potential interest [27].

6.5 Conclusion

In this study, the biocompatibility of novel drug delivery yarns hybridized with existing surgical sutures was characterized. These new drug delivery sutures could be further optimized to enhance autologous recellularization of implanted conduits and reduce suture-line scarring and calcification. The drug delivery yarns used in this study do not

alter the properties of existing surgical sutures and promote tissue suture integration. These yarns could also be used as a potential scaffold for the clinical reconstruction of tissues. Further experiments should be performed using a small animal vascular osteogenesis model to evaluate other putative therapeutic targets and adjust the dose/timing effects for enhanced wound healing and reduced calcification.

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6.7 Tables

Table 6.1: Drug delivery yarn formulations

Formulation	DXM	NOG	PLLA	PLGA	PVP	PEG	Yarn Pattern (left to right)
A	5% (25 mg)	–	475 mg	25 mg	5 mg	–	BAAAAAB (6-ply)
B	3% (30 mg)	–	800 mg	100 mg	100 mg	–	–
C	–	0.0220% (50 µg)	212 mg	11 mg	–	2 mg	DDCD (4-ply)
D*	–	0.0125% (50 µg)	–	400 mg	–	–	DDDD (4-ply)

*2% (v/v) Span-85 was added prior to spinning

Table 6.2: Transesophageal echocardiography measurements at 10 weeks post-implantation for juvenile sheep receiving a pulmonary valve in the right ventricular outflow tract

n	Treatment Group	Annulus Diameter (mm)		Mean Gradient (mm Hg)		Peak Gradient (mm Hg)		Cardiac Output (L min ⁻¹)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
6	Treated	17.90	0.17	3.33	0.55	5.33	0.74	5.28	0.11
4	Untreated	17.78	0.18	2.50	0.14	5.25	0.13	4.95	0.07

Table 6.3: Differential gene expression of treated anastomosis/untreated anastomoses

Gene	Log ₁₀ (Relative Quantification)
INFLAMMATION/ WOUND HEALING	
collagen type I, alpha	−0.802
smooth muscle actin	0.167
transforming growth factor-beta 1	−0.034
transforming growth factor-beta 2	−0.373
transforming growth factor-beta 3	−0.147
tumor necrosis factor-alpha	−0.869
VASCULAR OSTEOGENESIS	
bone morphogenic protein-2	−0.499
bone morphogenic protein-4	0.009
decorin	0.048
matrix metalloproteinase-2	−0.309
osteocalcin	−0.755
osteopontin	−0.665

6.8 Figures

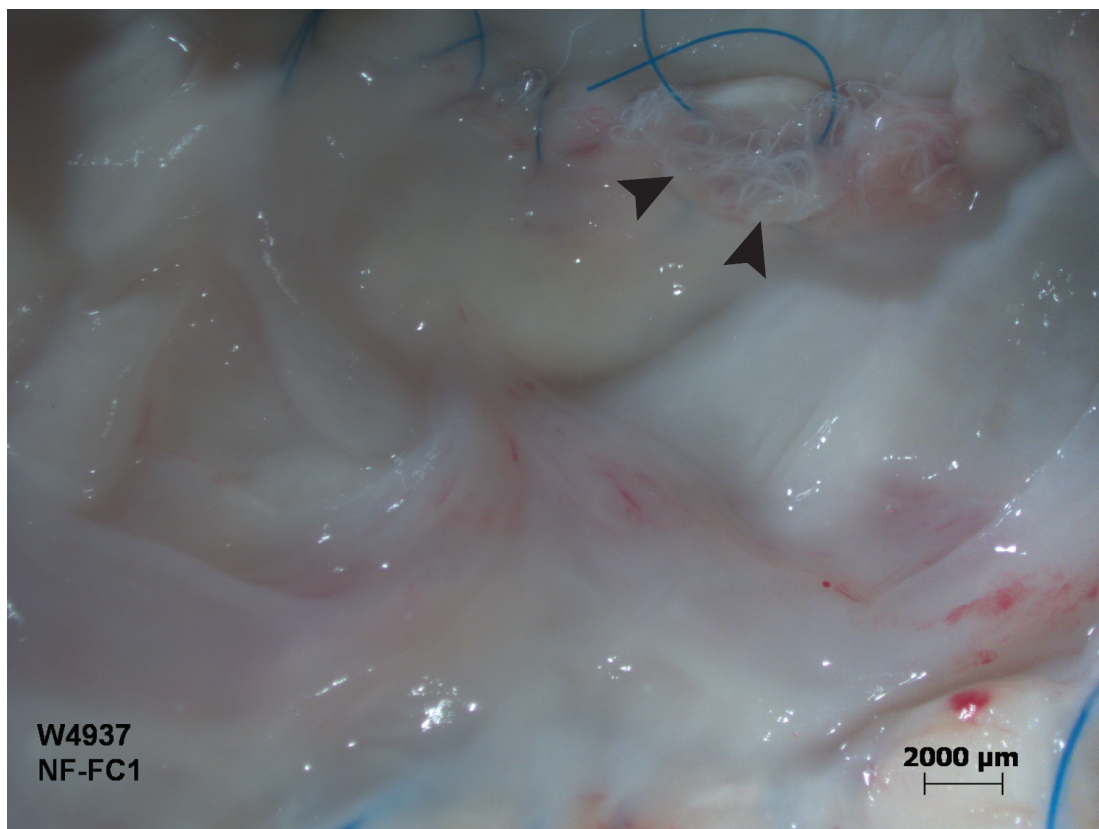


Figure 6.1: Drug delivery yarns are intact after 20 weeks implantation. Photographic image of explanted cryopreserved conduit. Arrows denote wet spun yarns present in distal anastomosis of a tricuspid pulmonary valve implanted in the right ventricular outflow tract of a juvenile sheep.

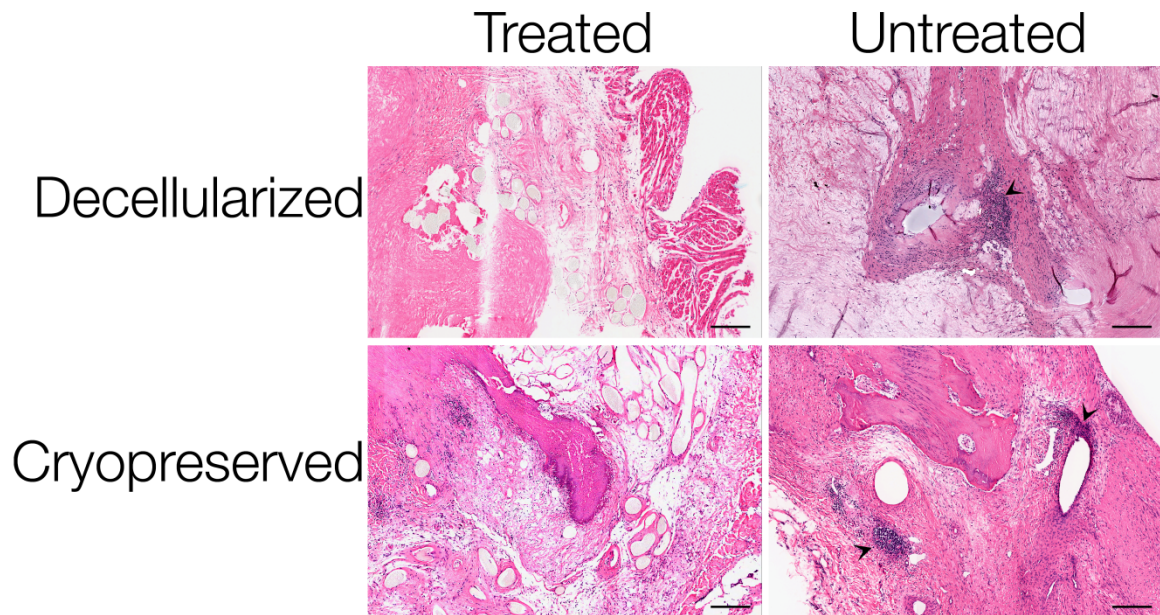


Figure 6.2: Remodeling, inflammation, and infiltration after 20 weeks implantation is different with treatment group. Selected images of suture anastomoses processed for H&E histology. Arrows denote tissue areas with multinucleated cells. Scale bar, 200 μm .

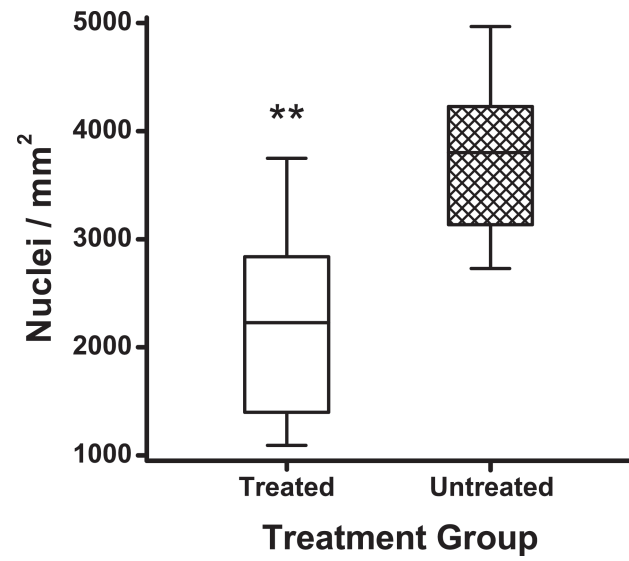


Figure 6.3: The number of cells surrounding implanted sutures is statistically different with treatment group. Box plot distributions comparing treatment groups showing the number of nuclei in tissues surrounding implanted sutures. Tops and bottoms of boxes indicate interquartile ranges, bands indicate means, and whiskers extend from 5th to 95th percentiles. $**p < 0.01$.

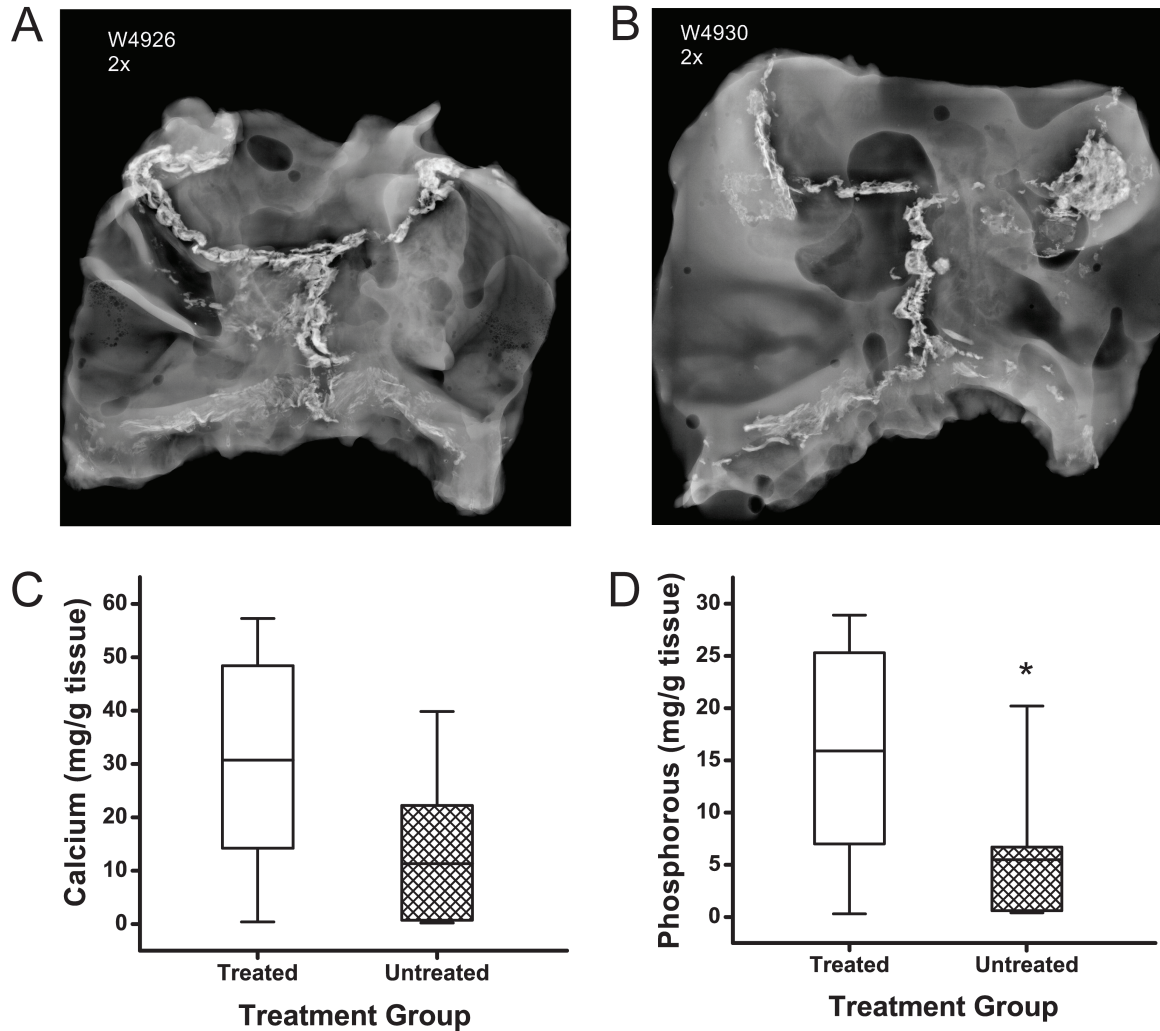


Figure 6.4: Suture-lines calcify over 20 weeks implantation. Representative radiographic images of (A) treated and (B) untreated bicuspidized cryopreserved valves showing calcifications along all suture lines. ICP-OES detection of (C) calcium and (D) phosphorous within the sutured tissues of distal and vertical anastomoses are presented by box plots illustrating the variability among treatment groups. Tops and bottoms of boxes indicate interquartile ranges, bands indicate means, and whiskers extend from 5th to 95th percentiles. $*p < 0.05$.

Chapter 7

Conclusions and future directions

There were four primary objectives in this thesis: (1) to develop novel wet spinning methods to fabricate drug-eluting fibers; (2) to encapsulate and deliver therapeutic molecules at biologically relevant rates using the methods developed in objective (1); (3) to evaluate the intrinsic material properties of wet spun delivery systems designed in objective (2) as they apply to biomedical and tissue engineering applications; and (4) to assess the potential of bioengineered fibers for use as drug delivery sutures *in vivo*. These goals were created to determine the therapeutic potential of wet spun fibers in the design of controlled release drug delivery systems and multifunctional scaffolds/biomedical implants.

Chapter 1 provided a review of the four common methods of polymeric fiber fabrication: melt spinning, dry spinning, wet spinning and electrospinning. The chapter discussed the fabrication conditions for spinning polymers into monofilaments, and outlined the advantages and limitations of each method in the design of multifunctional delivery systems. As highlighted in Chapter 1, wet spinning was selected for this thesis because this technique readily lends itself to drug delivery technologies and produces fibers within the size ranges of surgical sutures. The chapter discussed the current applications of wet spun fibers and identified the need for new wet extrusion methods for creating

polymeric fibers and a better understanding of the effects of drug encapsulation on the mechanical properties of filaments.

In Chapter 2, a new wet extrusion method for the fabrication of self-assembled polymeric scaffolds with fiber diameters was described. The most striking advantage of this wet extrusion method over other wet spinning techniques is the one-step formation of nonwoven self-assembled microfiber scaffolds with tunable fiber diameter, morphology, and drug release kinetics. This study points to some correlation between fiber size and morphology and both the precipitation strength of polymer solutions and the kinematic viscosity of the stirring nonsolvent coagulation bath. Narrow fiber distributions with significantly smaller mean diameters could be achieved by lowering the spin dope concentration, reducing the difference between solvent and nonsolvent miscibility parameters, and increasing the shear forces imparted on polymer streams during gelation in the coagulation bath. As well, Chapter 2 demonstrated how useful this technique is for drug delivery. Two different drug encapsulation techniques were used to achieve immediate (50% of drug eluted after 5-day incubation) and prolonged (50% of drug eluted $\geq$ after 35-day incubation) delivery of heparin by altering the porous interconnectivity of polymeric microfibers.

Future directions for this project include studying the effects of spin dope preparation containing aqueous solutions on the porous interconnectivity of nonwoven self-assembled scaffolds. These studies should investigate the possibility of using micro-emulsions with different aqueous to organic phase ratios to change the porosity/porous interconnectivity of microfibers. Of particular interest would be the effects of porosity/porous interconnectivity on the release kinetics of multiple essential soluble and chemotropic factors that could be useful for specific therapeutic targets. For example, the delivery of vascular endothelial growth factor (VEGF) [1], and fibroblast growth factor (FGF) [2], could be beneficial for tissue engineering arterial substitutes.

Chapter 3 presented a processing technique for the fabrication of a stable fiber delivery system capable of providing both controlled release and mechanical support. In this study, the dynamic mechanical properties of drug-eluting wet spun microfibers were examined *in vitro*. While there are many methods for increasing the mechanical strength of fibers by physical manipulation through post-drawing techniques, this chapter discussed the fabrication of wet spun fibers where processing conditions alone enhanced fiber strength and stability. The choice of solvents, nonsolvent, and residence time during fabrication were found to result in solvent-induced crystallization (SINC) as judged by an increase in relative crystallinity and crystalline structure. The incorporation of dexamethasone, the model therapeutic selected for this study, led to further crystallization that ultimately increased the mechanical stability of wet spun filaments. Fibers with the highest drug loading were capable of linear release of up to 28% of total encapsulated drug after eight weeks *in vitro* with 97% tensile strength retention. The main finding in this chapter was that the addition of small hydrophobic drugs (<400 Da), such as dexamethasone, could be used to increase the mechanical integrity of filaments whose crystallinity is increased as a result of fabrication.

Future directions for this project include a meta-analysis evaluating crystallinity and crystalline structures of wet spun fibers fabricated using different processing conditions. More specific correlations between the processing conditions and structure of precipitating polymer chains can be made by determining the precise wet spinning conditions needed to induce SINC. Experimental data can also be compared to the tensile properties of wet spun fibers with and without SINC to further establish a relationship between crystalline structure and mechanical strength.

Chapter 4 demonstrated that the size of encapsulated proteins has an effect on the mechanical strength and release kinetics of wet spun fibers. Both strength and elongation of filaments were found to significantly decrease with increased protein molecular weight. This response was mainly due to polymer chain disruption during filament precipitation. Characterization of the material properties showed that the

strength and elongation of wet spun fibers was material-specific, and that amorphous PLGA fibers encapsulating the highest molecular weight protein (66 kDa) had superior tensile strength retention and ductility compared to PLLA fibers loaded with the same protein. The release of encapsulated proteins was also found to be molecular weight-dependent; large proteins had prolonged release profiles compared to smaller molecular weight proteins. Further analysis with the Korsmeyer-Peppas kinetic model determined the mechanism of drug release was dependent on Fickian diffusion. Chapter 4 highlighted the importance of designing small molecular analogues to replace growth factors with large molecular weights for the design of multifunctional wet spun fibers.

An underlying goal of this thesis was to break down the complexity of wet spinning into the processes contributing to mechanical strength and drug delivery attributes, and to begin to recombine these processes to develop multifunctional fiber platforms towards the design of drug-eluting sutures. Chapter 5 primarily focused on understanding what wet spinning conditions are optimal for the development of drug delivery sutures, taking into consideration the lessons learned in Chapters 3 and 4. The experiments discussed in Chapter 5 showed that hydrophilic polymers and excipients tuned the release kinetics of dexamethasone within the therapeutic levels of drug treatment, and that the biological activity of eluted drug was preserved up to 56-day incubation. Chapter 5 presented three different processing methodologies (as-spun, solution-drawn, and post-drawn), further demonstrating the complexity of designing hybrid wet spun fiber platforms. In addition, a bench top technique to engineer monofilaments into multifilament yarns was created to increase the load-bearing strength of individual wet spun fibers. A mathematical equation derived to predict the dexamethasone release profiles of multifilament yarns using the experimental release profiles of individual filaments was also verified in Chapter 5.

Future directions of this work point to the optimization of wet spun fiber delivery platforms for the design of tissue-engineered scaffolds, as well as the investigation of incorporating yarns into more complex geometric superstructures suited for biomedical

applications. It is well recognized that the design of tissue engineered scaffolds and substrates to meet the physiological function of tissues cannot be achieved without adequate consideration of mechanical and biological factors [3]. It is also likely that the combination of both factors, rather than any single factor, will ultimately guide the translation of tissue engineering to the clinical arena. To determine the full capacity of wet spun fibers in such applications, further studies examining the cellular responses to fibers and therapeutic potential of eluting essential factors to enhance the repair and regeneration of tissues in *in vivo* animal models should be performed.

Insights into the general processing conditions resulting in wet spun filaments with diverse surface structures were also elucidated in Chapter 5. Manipulation of polymer-solvent and solvent-nonsolvent phase separation parameters demonstrated the ability to make micron-scale alterations to the surface topographies of wet spun filaments. Although unreported in Chapter 5, we also showed that polymer-polymer phase separation parameters could be used to create multiconstituent wet spun fibers with spatially distinct morphologies (**Appendix A, Figure A.4**). Besides the phase separation contributions to morphology, we also looked at the drug delivery characteristics of phase-separated fibers (**Appendix A, Figure A.5**). Different types of fibers with topographical cues and dual drug delivery systems should be incorporated into the designs of fiber based scaffolds and biomedical implants for further evaluation.

The work from this thesis culminated in the design of a surgically implantable drug delivery suture for cardiovascular applications as discussed in Chapter 6. The application of multifilament wet spun yarns as drug delivery sutures was evaluated in an established *in vivo* ovine model for calcification. Specifically, Chapter 6 studied the potential of dexamethasone and noggin as local drug delivery interventions to reduce unwanted host immune responses at the surgical implantation sites of tissue engineered constructs. Braided drug eluting sutures were woven within the anastomoses of tissue engineered heart valves implanted in a large animal model. Calcific responses were not substantially different with the therapeutics tested, suggesting that these molecules do

not play a large role in suture-driven tissue mineralization, or that the dose of dexamethasone and noggin was not substantial enough to slow tissue mineralization. The main finding of this chapter was that anastomoses with drug treatment had significantly reduced cellular infiltrates surrounding implanted suture materials.

Despite the usefulness of the yarn methodology developed in Chapter 5 for the scale up of monofilaments into braided surgical sutures in Chapter 6, there were some limitations. As previously described, the surgical procedures used for the implantation of tissue engineered heart valves in the ovine model resulted in filament fraying, and in some cases, fracture. Consequently, only one load-bearing suture was implanted in these studies. However, the knowledge gained during surgical handling and implantation is invaluable for the design of future prototypes.

Future directions point to studying the dosing and timing regimes of multiple therapeutic targets in drug delivery interventions for suture-driven fibrocalcification. Other specific targets of potential interest for therapeutic modulation of wound repair are osteopontin and platelet derived growth factor (PDGF), since inhibition of these proteins have recently been implicated in accelerated wound healing and reduced scarring [4]. A small animal model for studying the fibrotic and calcification responses to tissue engineered constructs could also be developed. The technologies developed in the scale up of single wet spun filaments into drug eluting sutures could also be used as a platform for the design of other specialized classes of suture delivery systems from the principles learned in Chapters 2-6.

In conclusion, this thesis focused on a classical biomedical engineering project – one that linked drug release and hybrid biomaterials to address a real clinical problem that requires sophisticated solutions. The principles learned in this thesis can be used to incorporate hydrophobic/ hydrophilic drugs into polymeric fibers without destroying the mechanical integrity of the filaments. While the incorporation of drugs may at first appear to be a simple process, we discuss how the choice of solvent and drug/ polymer

interactions determines the molecular morphology of wet spun filaments, which can be used to enhance the final strength of precipitating fibers through solvent-induced crystallization (SINC). This is an integral concept; if we choose the right hydrophobic drug to encapsulate, we can achieve release for a prolonged period of time (months to years) and maintain filament strength. The breakdown of the complex wet spinning process contributes to a deeper understanding of the phase separation fundamentals that lead to the gelation and transformation of polymeric streams into solid filaments during immersion precipitation. By better understanding the intricacy of the wet spinning technique, this thesis demonstrates how to engineer multifunctional polymeric fibers, ones that provide structural support and simultaneously delivery of drugs for a number of biomedical and tissue engineering applications.

It is my sincere hope that the lessons learned in this thesis could one day contribute to the development of a drug delivery suture to improve the surgical outcomes of cardiovascular constructs implanted in pediatric patients who suffer from congenital heart defects and other diseases.

7.1 References

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Appendix A

Preparation and characterization of binary phase microfibers using a novel one-step wet extrusion method

We used our knowledge of polymer-polymer phase separation to create a new method for the fabrication of spatially distinct microfibers. In this method, 20% (w/v) poly(L-lactic acid) (PLLA) and poly(lactic-co-glycolic acid) (PLGA) were dissolved in dichloromethane (DCM) at 1:1 ratio. Under these specific conditions, the solubility of PLLA in the DCM/PLGA decreases, and the polymer begins to precipitate out of solution, or phase separate. **Figure A.1** shows a typical optical image of a phase separated PLLA:PLGA solution. PLLA spherulites represented by the maltese cross patterns are visible within a continuous PLGA phase. While the phase separation of 1:1, 1:2, and 1:3 PLLA:PLGA solutions was observed under cross-polarized optical microscopy, only the 1:1 spin dope solution was suitable for the formation of continuous monofilaments within the spin bath.

Differential scanning calorimetry (DSC) was used to confirm the separation PLLA and PLGA in wet spun microfibers. DSC thermograms exhibited the presence of two distinct glass transitions in the first heating scan of PLLA:PLGA (1:1) microfibers (**Figure A.2**). The presence of two glass transitions, each representative of PLLA and PLGA

controls, is evidence of a phase separated blend. If the solution were a miscible blend of PLLA and PLGA, the thermal properties would be between those of the two unblended polymers. No apparent differences in the C-S morphology of binary phase microfibers were observed in comparison to PLLA and PLGA microfiber controls (**Figure A.4**). Since PLLA and PLGA microfibers display similar C-S morphologies, it is hard to distinguish variations in phase separated blends. Thus, we evaluated other phase separated solutions of PLLA and polycaprolactone (PCL), since these polymers are known to easily phase separate. The morphology of microfibers spun from 1:3, 1:1, and 3:1 PLLA:PCL phase separated spin dope solutions is shown in **Figure A.4**. PLLA:PCL blends show evidence of spatially distinct microfibers.

Unlike the previous work done by our lab on the fabrication of double-walled microspheres, the composite microfibers fabricated in this study did not exhibit a distinct core-shell morphology. Partial separation is likely due to differences in fabrication technique. Double-walled microspheres are made by solvent removal and solvent evaporation, processes that can take several hours before microspheres are fully precipitated. Wet spinning by phase inversion is nearly an instantaneous process governed by solvent and nonsolvent miscibility. Hence, phase separated spin dope solutions do not fully separate during microfiber precipitation and PLLA regions are solidified within the continuous PLGA phase. Our wet spinning technique could be further optimized to achieve microfiber composites with core-shell morphology by altering solvent/nonsolvent miscibility parameters, residence time, and polymer precipitation strength.

A model hydrophilic protein, bovine serum albumin (BSA) was encapsulated within wet spun filaments using the cryogenic emulsion technique described in Chapter 4. BSA was added to the organic phase of PLLA/DCM solution, flash frozen in liquid nitrogen, and lyophilized overnight. The resulting polymer cake was then reconstituted in DCM. PLGA was dissolved in the PLLA/DCM/micronized BSA solution, and wet spun by phase inversion. The release profile of BSA-loaded PLLA:PLGA (1:1) microfibers in

comparison to PLGA and PLLA controls is shown in **Figure A.5**. PLLA:PLGA (1:1) had reduced burst effect in comparison to microfibers spun from PLLA and PLGA only up to 35-day incubation. Microfibers fabricated from the phase separation of binary blends may be important for the delivery of drugs with narrow therapeutic ranges. As well, the design of spatially distinct polymeric microfibers offers the ability to develop dual drug delivery systems for control over the spatial and temporal release of therapeutics. These studies emphasize yet another degree of hierarchy available in the design of wet spun fiber delivery platforms.

A.1 Figures

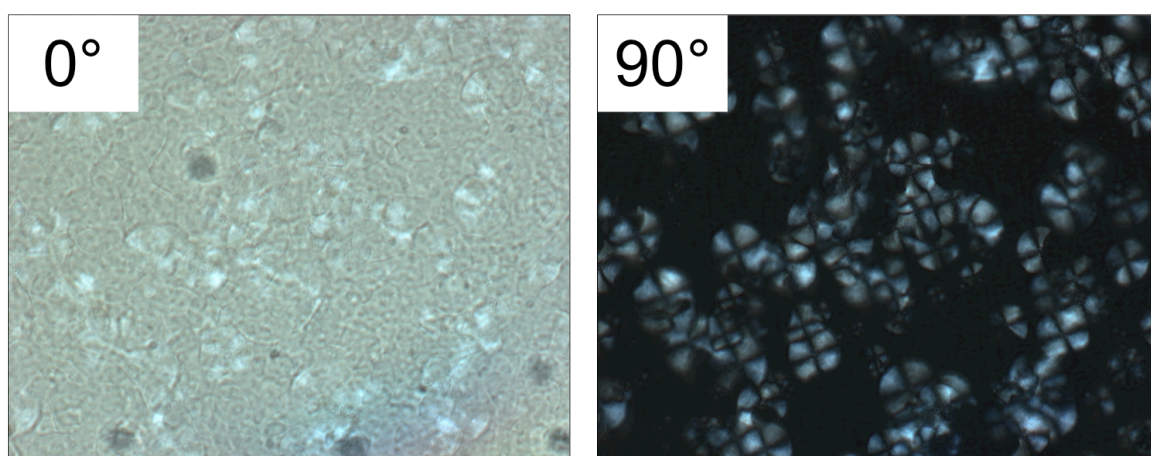


Figure A.1: PLLA/PLGA solutions phase separate. Cross-polarized optical micrographs of a 20% (w/v) 1:1 PLLA to PLGA film-cast polymer solution captured using a 20x objective. Maltese cross patterns present at 90° show PLLA droplets within a continuous PLGA phase.

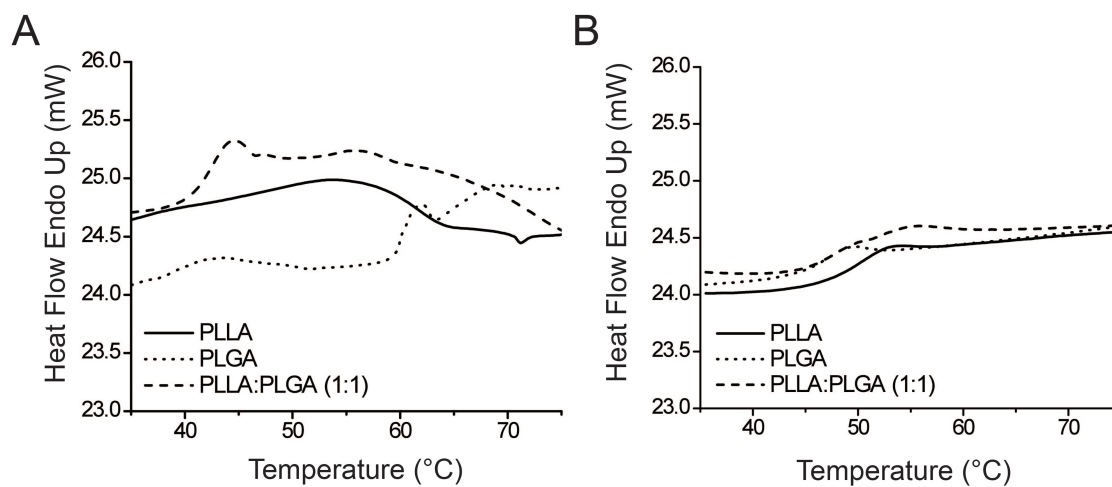


Figure A.2: Binary phase microfibers have two distinct glass transition temperatures. DSC thermograms from the (A) first and (B) second heating scan of microfibers wet spun from 20% polymer spin dope solutions. The presence of two glass transitions, each representative of PLLA and PLGA controls, is evidence of a phase separated polymer microfiber.

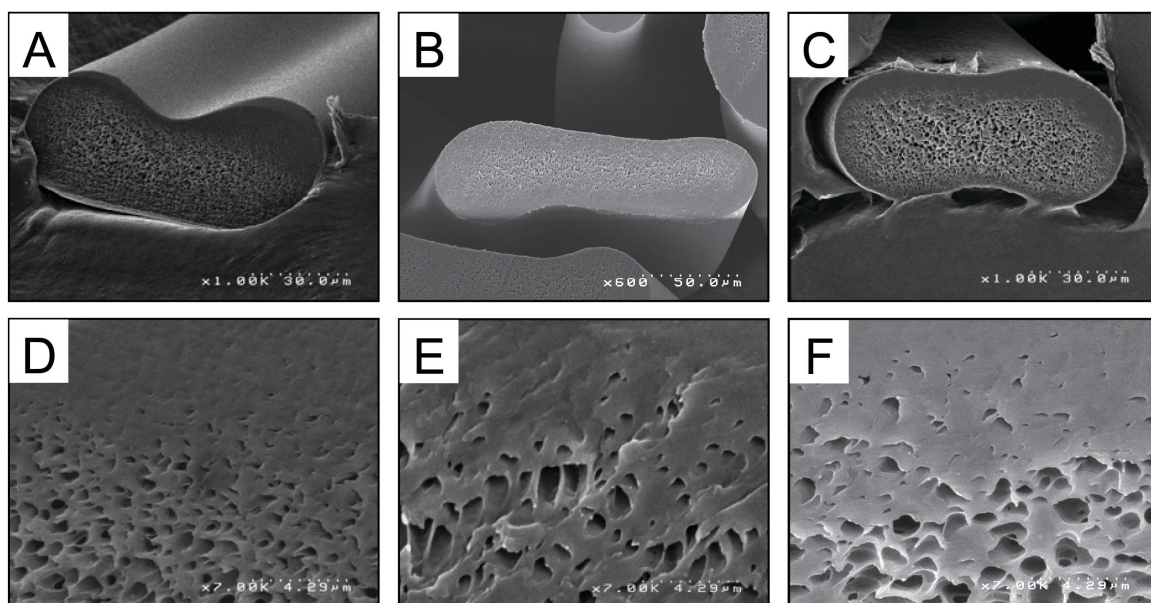


Figure A.3: The morphology of phase separated PLLA:PLGA microfibers is similar to PLLA and PLGA controls. Scanning electron micrographs of cross-sectioned PLLA (A, D), PLGA (B, E), and 1:1, PLLA:PLGA (C, F) microfibers. Scale bars, 30 μm (A, C), 50 μm (B), and 4.29 μm (D, E, F), respectively.

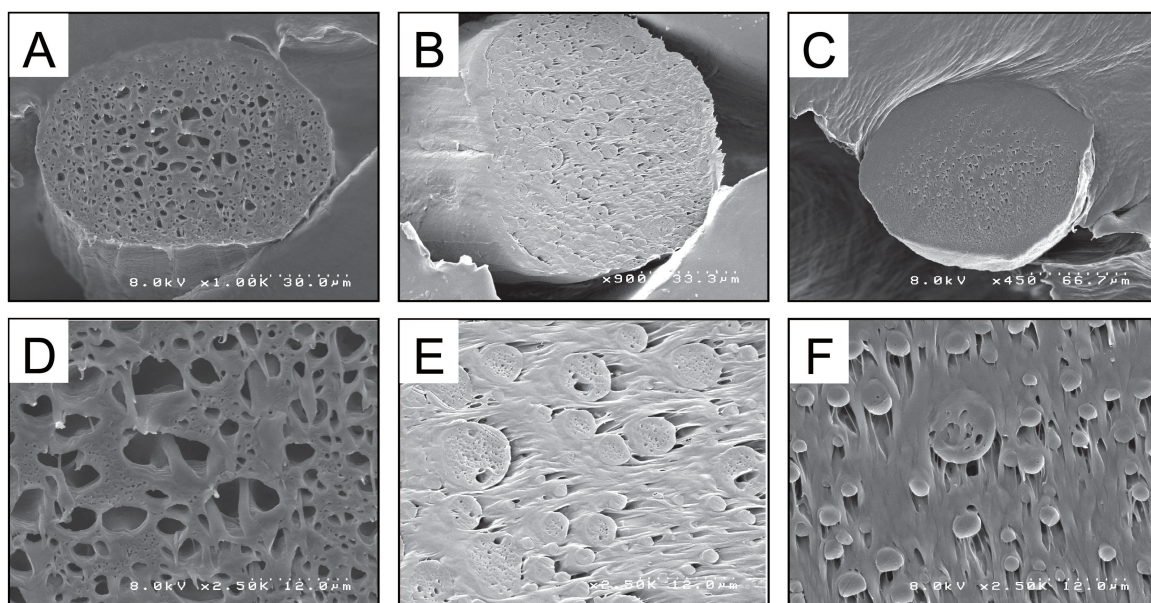


Figure A.4: The morphology of PLLA and PCL microfibers are distinct. Scanning electron micrographs of cross-sectioned 3:1 PLLA:PCL 50K (A, D), 1:1 PLLA:PCL 80K, and 1:3 PLLA:PCL 80K microfibers. Scale bars, 30 μm (A), 33.3 μm (B), 66.7 μm (C), and 12 μm (D, E, F), respectively.

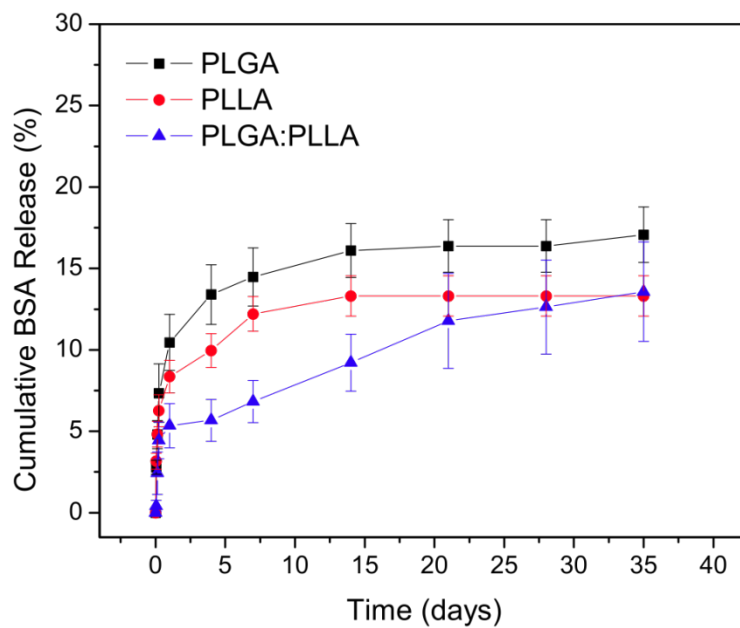


Figure A.5: Cumulative release kinetics of PLGA, PLLA, and PLLA:PLGA (1:1) microfibers loaded with bovine serum albumin (BSA). Fibers with PLLA:PLGA (1:1) show reduced burst effect in comparison to microfibers spun from PLLA and PLGA only. Mean $\pm$ S.D. are represented ($n = 3$).