

December 2018

Biodegradable and Biocompatible Silk Fibroin for Optical Applications

Corey Bryce Malinowski
cbmalinowski@hotmail.com

Follow this and additional works at: <https://digitalscholarship.unlv.edu/thesesdissertations>



Part of the [Biomedical Engineering and Bioengineering Commons](#), and the [Mechanical Engineering Commons](#)

Repository Citation

Malinowski, Corey Bryce, "Biodegradable and Biocompatible Silk Fibroin for Optical Applications" (2018). *UNLV Theses, Dissertations, Professional Papers, and Capstones*. 3432.
<https://digitalscholarship.unlv.edu/thesesdissertations/3432>

This Thesis is protected by copyright and/or related rights. It has been brought to you by Digital Scholarship@UNLV with permission from the rights-holder(s). You are free to use this Thesis in any way that is permitted by the copyright and related rights legislation that applies to your use. For other uses you need to obtain permission from the rights-holder(s) directly, unless additional rights are indicated by a Creative Commons license in the record and/or on the work itself.

This Thesis has been accepted for inclusion in UNLV Theses, Dissertations, Professional Papers, and Capstones by an authorized administrator of Digital Scholarship@UNLV. For more information, please contact digitalscholarship@unlv.edu.

BIODEGRADABLE AND BIOCOMPATIBLE SILK FIBROIN FOR OPTICAL APPLICATIONS

By

Corey Malinowski

Bachelor of Science in Engineering – Mechanical Engineering
University of Nevada, Las Vegas
2017

A thesis submitted in partial fulfillment
of the requirements for the

Master of Science in Engineering – Mechanical Engineering

Department of Mechanical Engineering
Howard R. Hughes College of Engineering
The Graduate College

University of Nevada, Las Vegas
December 2018



Thesis Approval

The Graduate College
The University of Nevada, Las Vegas

November 13, 2018

This thesis prepared by

Corey Malinowski

entitled

Biodegradable and Biocompatible Silk Fibroin for Optical Applications

is approved in partial fulfillment of the requirements for the degree of

Master of Science in Engineering – Mechanical Engineering
Department of Mechanical Engineering

Hui Zhao, Ph.D.
Examination Committee Chair

Kathryn Hausbeck Korgan, Ph.D.
Graduate College Interim Dean

Kwang Kim, Ph.D.
Examination Committee Member

Jaeyun Moon, Ph.D.
Examination Committee Member

Shengjie Zhai, Ph.D.
Examination Committee Member

Hui Zhang, Ph.D.
Graduate College Faculty Representative

Abstract

This study presents nanopatterned silk fibroin films that were fabricated using soft lithography and nanoimprinting to replicate patterns from diffraction gratings. These film's optics were analyzed based on their light scattering potential as well as their transmittance and transmission haze using a laser light, spectrometer, and UV-Vis spectrophotometer, respectively. The patterned fibroin films all displayed similar light scattering patterns to their master patterns with some transmission haze. When using the spectrometer to measure samples, those made without any nanostructure displayed transmission of 90% and over, while those with patterns depended on the structure used. The denser a structure, like the mesh structure used, resulted in lower transmission (~70%), while those with a less dense linear structure displayed higher transmission (~80%). This agreed well with transmission measurements of patterned polydimethylsiloxane (PDMS) molds created to fabricate the patterned films. When using the more complex spectrophotometer device to measure the transmission, the light transmitted by all films was found to be much higher, over 90%. This is because the spectrophotometer can measure the total transmission, while the spectrometer apparatus using optical fibers can not collect light that is scattered beyond the diameter of the fiber. For the transmission haze, samples displayed high haze of over 65% across a 400-850 nm range, with nanostructures adding additional haze, up to 70% with a mesh structure. Based on this analysis, a new direction is open to combine transparent fibroin films with high haze with either solar cells to act as an anti-reflection (AR) coating with its light scattering abilities or as a short-range, bio-dissolvable implantable device for photodynamic therapy (PDT) to act as an optical diffuser by combining the film with a degummed silk fiber that can act as an optical waveguide. These technologies can give new arrangements for bio-photonics devices to be used in photovoltaics (PV) or medical treatments that have the advantage of being totally biodegradable and biocompatible making them safe for the environment and human bodies.

Acknowledgements

I would like to thank my advisor and mentor for my Bachelor and Master studies, Dr. Hui Zhao of UNLV. He was always available in his office whenever assistance was needed and kept an open door and open mind to all my concerns. Under his guidance, he helped steer me into a research field I would never have seen myself working in and gave me plenty of room to grow as a student, researcher, and professional. Thanks to his mentorship, I have significantly improved my professional writing, critical reading, and problem-solving skills from when I started to work with him as an undergraduate student. He allowed me to take on the thesis paper as my own and provided necessary assistance when needed and I always left meetings with him with a renewed sense of confidence thanks to his brilliance and calm temperament.

Also, I would like to thank those who played significant roles in helping provide their expertise in refining this work and contributing valuable time and effort to it for many months. Dr. Shengjie (Patrick) Zhai of UNLV has been on the front lines of the experiments performed in this work and has had his hands in every step taken to move this research towards its finish. His understanding of optics and the electrical aspects of this project proved invaluable in the design, execution, and presentation of the experiments. He provided the tools for this project to succeed by making sure I had all the materials necessary to complete this project. Further, I would like to thank Fengjie He for her contributions and insight into the experimental work done for this project. She provided a biomedical engineering background that helped my understanding of the biology side of this project.

Finally, I would like to express my profound gratitude to my parents, friends and, my girlfriend for their unwavering support of my work and studies. Their love and desire for me to succeed has provided me a sense of comfort that allowed me to continue pushing even when I felt discouraged.

Table of Contents

Abstract.....	iii
Acknowledgements.....	iv
List of Tables.....	vi
List of Figures.....	vii
Chapter 1: Introduction.....	1
Chapter 2: Silk Fibroin Materials Fabrication.....	5
Chapter 3: Fabricating Nanostructured Silk Films.....	14
Chapter 4: Optical Characterization.....	23
Chapter 5: Conclusion.....	33
References.....	35
Curriculum Vitae.....	37

List of Tables

Table 1: Thickness Measurements of Fibroin Films.....	25
---	----

List of Figures

Figure 1: SEM Images of the Linear (top left and right) and Mesh (bottom left and right) diffraction gratings enhanced with ImageJ.....	16
Figure 2: PDMS mold with linear (left) structure and mesh (right) structure.....	17
Figure 3: Patterned 1.5 x 1.5 cm 5 wt% silk fibroin films with the linear (left) and mesh (right) structures....	18
Figure 4: Plasma Cleaner PDC-32G.....	19
Figure 5: Linear patterned PDMS stamp.....	20
Figure 6: Incomplete patterning of a silk-coated slide.	21
Figure 7: Nanoimprinted silk film with significant dissolved sections.	22
Figure 8: Light scattering of master, PDMS, and fibroin films for the laser light test	24
Figure 9: Spectrometer Experiment Set-up.....	27
Figure 10: Spectrometer transmission measurements.....	28
Figure 11: Spectrophotometer transmission (left) and transmission haze (right) measurement.....	30

Chapter 1: Introduction

1.1 Silk Fibroin and Conventional Applications

Silk materials have recently begun gaining popularity as a cutting-edge biopolymer research field. The native silk fibroin solution can be formed into numerous material formats, like a thin film, hydrogel, sponge, 3D scaffold, 3D printing inks, microspheres and nanoparticles. The biocompatibility, as well as the robust mechanical properties it boasts make it attractive to those studying biopolymers as an alternative to conventional implantable medical devices. Notably, the Kaplan group at Tufts University have done much of the fundamental research on this protein, leading studies and collaborating with groups from around the world [1]–[3]. Their studies focused initially on silk material properties and its uses in tissue engineering and drug delivery [4], [5]. In the first study, they use integrin, recognition sequences, parathyroid hormone (PTH), and a modified PTH inside a silk fibroin film on osteoblast-like cells to showcase calcification and mineralization of bone *in vitro*. They found the recognition sequences stimulated this mineralization the best, and that this silk fibroin material was able to induce this bone growth. In the second study, silk fibroin films were used as an encapsulant for release of enzymes like lysozyme or horseradish peroxidase. Due to the higher crystallinity from their methanol treatment, they were able to exhibit controlled release of the enzymes, which has been utilized by others for different treatments; such as solid cancer tumors [6] and anticancer drug epirubicin [7]. These later studies, began to use other material formats that they found to be more effective in the distribution of the drug than simply an integrated silk fibroin film, such as silk fibroin nanoparticles.

Mainly, the silk fibroin hydrogel has gained the most traction as an implantable medical device. Due to its easy fabrication from solution by sonication [8] or vortexing [9], its tunable compressive and tensile modulus which can reach up to 3.0 and 2.5 MPa [10], and its

dissolvable property, it has become very popular in cell encapsulation and controlled drug delivery studies. For example, a group from the Department of Textile Technology at the Indian Institute of Technology studied a bioprintable silk fibroin-gelatin hydrogel for cell encapsulation [11]. Here, they developed a bioink consisting of silk fibroin and gelatin that were physically crosslinked by sonication. This bioink was 3D bioprinted with mesenchymal progenitor cells from human nasal inferior turbinate that could fabricate 3D tissue constructs. Using this unique strategy, combining a new material like silk fibroin with a robust biopolymer like gelatin offered another path to direct printing for customizable 3D structures for patients. This shows the versatility of this material and its ability to invent new avenues to approach stagnant research fields.

1.2 Silk Fibroin, Thin Films, and Optics

The focus of this work is not on the conventional applications of silk fibroin thin films, but ones involving its optical properties. Silk fibroin has had work done in the field of electronics and optoelectronics [12]. Due to its flexibility, silk fibroin is seen as a possible substitute to some materials such as silicon. Moreover, a group from the Institute of Functional Nano & Soft Materials (FUNSOM) at Soochow University, Suzhou were able to introduce silver nanowires into native silk fibroin solution, making silk fibroin films that are conductive, and due to their transparency can be used as a substrate in perovskite solar cells [13]. The low-cost of organic electronics is gaining ground in the field of photovoltaics (PV), and solar cells in clothing is a large next step in wearable electronics. This group achieved a conductivity in the silver nanowire/silk fibroin layer of $\sim 11.0 \Omega/\text{sq.}$ and a transmittance of $\sim 80\%$ in the visible light range. The application to clothing would be astounding due to the device's lightweight and ability to retain the conductivity even after being bent and unbent 200 times. Compared to indium tin oxide or a synthetic plastic substrate, they lost their conductivity at much less rigid bending. They even mention that large-scale roll-to-roll processing.

Another creative optical application using silk fibroin films is in an optical humidity sensor [14]. This team developed a sub-micron silk fibroin film by spin coating that retained a high sensitivity to humidity. Most photonic crystals have nanostructures that are difficult to fabricate, but with the silk fibroin solution, the fabrication is simplified. They report a color variation induced by de-bonding of hydrogen bonds in the fibroin polymers in as little as 5 s which is much faster than current humidity sensors that require multiple layers. With these results they propose applications in colorimetric detection of humidity akin to standard pH paper, where one could easily know the environment humidity without use of a formal detector or device. Also, they propose its use in anti-counterfeit labeling by applying a pattern by ion sputtering that is released when exposed to water vapor.

Silk fibroin can also be used to guide light. As the Sun group from Aalto University in Finland and also the Kaplan group demonstrates, degummed silk fibers as well as printed silk ink that can act as optical waveguides [15], [16]. In the former study, they investigate how a single degummed fiber can be used to guide light over 3 mm with an average of 2.8 dBmm^{-1} . They found small torsional twisting and defects in the fiber did cause some scattering losses, so for now they only recommend this waveguide in short distances. Such applications could be in photodynamic therapy (PDT) or any optical therapy and embedded in living tissue for medical imaging. The latter study involves preparing a concentrated aqueous silk fibroin solution of 28-30 wt% to serve as the bioink. The wave guide is extruded through a syringe on a three-axis micropositioning stage that was controlled by a customized software, where they could create arbitrary geometries. This helps expand the use of silk fibroin solution to create complex geometries for high quality optical waveguides where the losses when propagating light were between $0.25\text{-}0.81 \text{ dBcm}^{-1}$, where curved waveguides had a higher loss than straight ones.

The development of thin films for this work were based on already published fabrication techniques [17]–[19]. These groups have developed methods to fabricate regenerated silk fibroin solutions and thin films and patterned thin films. These methods are developed by

looking at classic nanofabrication techniques such as soft lithography and nanoimprinting. The motivation for the study, however is based on a work done by the University of Maryland, College Park's Department of Materials Science and Engineering. They created a nanostructured paper using TEMPO-oxidized wood fibers that demonstrated both ultrahigh haze and transparency [20]. Their paper exhibited a transparency of about ~96% and haze of about ~60% to be used for a substrate of a solar cell. They boast of the low material cost and high performance as well as its environmentally friendly property. They were able to achieve this thin film due to a high stacking density of the packed wood fibers of 1.14 g/cm^3 because of the TEMPO treatment. The goal for this study was to achieve a similar result, but incorporating nanopatterning to improve the light scattering even more, which sacrifices transparency. An optical diffuser can be used in many optical environments as previously presented. Substrates of solar cells, optical therapy, and sensors are all highly competitive research fields that can be further improved by eliminating harmful materials in their components.

Chapter 2: Silk Fibroin Materials Fabrication

2.1 Silk Fibroin Solution

2.11 Degumming

The silk materials fabricated follow standard procedures for silk fibroin-based materials with minor adjustments to some processes. *Bombyx Mori* silkworm cocoons were purchased from different Chinese suppliers. Degumming is the process in which the silk fibroin protein of the silk cocoon is separated from the gum-like protein sericin. For the first step in the degumming process, the desired mass of cocoons is measured on a medium weighing boat. Typically, 5 g of cocoons are used as the ratio of mass of cocoons to milliliters of sodium carbonate solution is 1:400. The silkworm cocoons must first be cut by scissors into small dime-sized pieces. Depending on the supplier, some cocoons still have remnants of the silkworm, as well as dust and dirt attached to the outer shell of the cocoon. Thus, the cocoons are cleaned by an ultrasonic cleaner filled with DI for 180 seconds. The cocoons are then scrubbed vigorously by hand to remove as much visible dust, dirt, and dried or damaged sections of the cocoons. Once cleaned, the cocoons are left to dry in an air dryer for about 4 hours.

After the cocoon pieces have fully dried, the sodium carbonate solution is prepared. If a mass of 5 g of cocoons was used, the volume of sodium carbonate solution shall be 2 L based on the previously mentioned mass to volume ratio. The desired molarity of the sodium carbonate solution should be 0.02 M, so 4.24 g of sodium carbonate is dissolved into a 2 L beaker of deionized (DI) water. This beaker is heated and covered with aluminum foil until a rolling boil is achieved. Then, the dry cocoon pieces are added to the solution and stirred with a glass stirring rod for 30 min. During this process, one should observe a distinct color change in the sodium carbonate solution from clear to a hazy yellow color. Also, one should notice the cocoon pieces beginning to lose their stiff shapes, become softer, and begin to aggregate

together. This means that sericin is successfully being removed from the cocoon pieces. After 30 min of degumming, the degummed silk fibers are removed from the solution and rinsed under a flow of ultrapure water. Finally, the sodium carbonate solution with the sericin dissolved into it is simply emptied into the sink.

The degummed silk fibers are then placed into a 1 L beaker of DI water with a magnetic stir bar and stirred for 20 min. Once then 20 min have passed the degummed fibers are rinsed again under a flow of water and the 1 L of DI water is replaced with new water. This is to ensure that all of sodium carbonate and sericin is removed from the degummed silk fibers. This process of rinsing and stirring in DI water is repeated two additional times, for a grand total of 4 rinses under ultrapure water and 3 times stirring in a 1 L beaker of DI water. Finally, the degummed fibers are left in the air dryer again and allowed to dry overnight. Most degumming procedures take around 2.5 to 3 hours time to complete.

2.12 Dissolving

After degumming, the silk fibers must be dissolved. There are a few options of solutions use a solvent to break down the fibroin protein, such as lithium bromide solution, HFIP, or formic acid. For the traditional procedure, 9.3 M LiBr solution is used as the solvent. Ultimately, the goal is to have the weight percent of the dissolved fibroin solution be 20% wt%/vol. Thus, the ratio comparing grams of degummed fibers to milliliters LiBr solution is 1:4.

The dried silk fibers are massed, and the measured mass is multiplied by 4 to discern the required volume of LiBr solution. **Eq. 1** represents this formally.

$$[MW_{LiBr} (g/mol)] [9.3 (mol/L)] (1 L / 1000 mL) [V_{LiBr} (mL)] = m_{LiBr} (g)$$

Eq. 1: Calculation of mass of LiBr, m_{LiBr} , required.

Here, MW_{LiBr} , the molecular weight of LiBr, is 86.85 g/mol, and the volume of LiBr, V_{LiBr} , is also calculated directly after massing the dried degummed fibers. Generally, after degumming there is about a 30% mass loss due to the sericin removal and removal of

contaminants on the silk samples such as the dust, dirt and pieces of the silk worms. Once the appropriate volume of 9.3 LiBr solution is prepared, the degummed fibers are packed tightly into two 5 mL Erlenmeyer flasks. Then, the 9.3 LiBr solution is poured over top and gravity allows for the solution to slowly dissolve the fibers. The dissolving fibers are placed into a 60°C water bath overnight to allow for full dissolution of the sample. Checking the samples the following day, the solution should be a little more viscous than water and should appear amber or orange. Occasionally there will be black fibers visible in the solution, but these are no problem as later processes help remove these contaminants.

2.13 Dialysis and Centrifugation

After dissolving the degummed fibers, the solvent must be removed to achieve an aqueous silk fibroin solution. The two Erlenmeyer flasks of dissolved fibroin solution should be emptied into a larger beaker for easy extraction by syringe. Then, the solution is inserted into a Slide-A-Lyzer dialysis cassette with a 3.5 kDA molecular weight cut-off (MWCO) through an 18-gauge needle. The volume of the dialysis bag or cassette should correspond to the volume of dissolved solution obtained so that there is efficient filtration of the solvent.

The dialysis cassette is placed into a 2 L beaker of DI water with a magnetic stir bar and is stirred slowly. If the speed is too high, the protein will precipitate out of the solution and this can result in lower concentration of the resultant pure fibroin solution. This is a similar mechanism to vortexing fibroin solution too fast which causes the protein to form large conglomerate molecules and appear as solid precipitates. Typically, during dialysis one can see solvent diffusing out of the bottom of the cassette into the water in the beaker. The water in the beaker must be changed a total of 6 times to be confident all the solvent has been removed. The water is changed after 1 h, 4 h, 8 h, 20 h, 32 h, and 44 h.

After 48 h of dialysis, the solution is removed from the cassette, again by an 18-gauge needle. In most of the experiments, the cassette's maximum volume was more than the total

volume of dissolved solution, so water diffuses into the cassette and results in a higher yield after dialysis. For example, with 5 g of cocoons initially, and after degumming there is 3.5 g of silk fibers which dissolves into about 12-15 mL of solution. After dialysis, the volume of this example would be around 40-50 mL due to the water entering the cassette. The solutions retrieved from the dialysis cassette is put into a 50 mL conical tube and sealed with Parafilm and placed into a refrigerator at 4°C until the centrifuge is ready.

Centrifugation is a vital step in purifying the protein solution. The low-temperature centrifuge (Eppendorf 5402R) must first be brought down to 4°C. Then, once at the correct temperature the solution is either split between two centrifuge tubes with equal volume in each or put into one centrifuge tube and a second tube is balanced with water. The tubes are placed into the centrifuge device and it is run at a rotational velocity of 12,700 g, or 9,000 RPM for 20 min. When removing the solution from the centrifuge device one should notice solid aggregates on one end of the centrifuge tube as well as any of the black-colored contaminants. The solution is removed from the tube leaving behind any of these aggregates or contaminants by transfer pipette or syringe and replaced into a clean centrifuge tube. This process is repeated two additional times. The result should be a transparent white colored solution that is slightly more viscous than water.

The pure fibroin solution should be stored in a tube, sealed, and kept at 4°C. The pure fibroin solution will experience gelation typically around 30 days after centrifugation but depending on if any premature formation of the secondary β -sheet structure of the protein has occurred. β -sheet crystal structures can form by shearing the solution after it has dissolved, which can happen when retrieving it out of a beaker or when removing it from the dialysis cassette. Also, one should take care not to shear it during transfer from centrifuge tubes during the centrifugation process. Premature formation of the secondary structure can cause gelation in as little as a 7 days or 14 days. Plus, the behavior and viscosity of the solution depends on

its age and how much of the amorphous β -sheet structure has formed. Over time, the solution will behave more like a hydrogel than a liquid solution which can be problematic for forming thin films and achieving repeatability in experiments.

2.14 Characterization of Silk Fibroin Solution

In this work, a few key characteristics of the fibroin solution should be determined. The first is the date the sample was purified by the centrifuge. As previously stated, the age has a significant impact on the resultant films so to best understand the result, one should know how long the silk fibroin solution has been stored for. Since the solution is in an unstable state, it will eventually begin to experience gelation and want to form an amorphous hydrogel. This form of the silk hydrogel is soft, weak, and brittle and tends to not serve any greater purpose for other protocols involving the silk hydrogel, such as drug delivery or cell encapsulation. This material form is usually discarded, and even though it can be re-dissolved into 9.3 M LiBr solution, the fibroin protein has already degraded and tends to not self-assemble as the original pure fibroin solution does.

The second characteristic, and probably the most important, is the concentration of the solution by weight percentage. This can be determined very easily by casting a known volume of fibroin solution onto a medium weighing boat and drying the solution at 60°C for about 3 hours. Once fully dried, the obtained silk film can be massed, then by multiplying this mass by 100% the weight percentage is obtained. Typically, the standard method described before will yield a fibroin solution with a concentration ranging from 7 wt% all the way to 9 wt%. The concentration obtained from the process can depend on many factors. For example, if the cocoons are boiled in the sodium carbonate solution for longer than 30 min in the degumming step, the concentration will be lower, and alternatively if the boiling time is reduced. Also, the type of cocoons used can affect the final concentration. Some cocoons used were of an orange hue from Thailand and they tend to contain more fibroin protein in them and are generally softer

to the touch than a typical Chinese silk worm cocoon. Otherwise, based on all the times the fibroin solution was made for this work, the concentration tended to vary based on experimental errors, such as solid participates forming in the dialysis cassette.

Additional characteristics that were measured for the fibroin solution were the initial pH value and the total dissolved solute (TDS) value. For the former, pH bears significance in understanding how close the fibroin solution is to its isoelectric point (pI) where one would observe a structural change. When performing experiments to form a silk hydrogel by reducing the pH value by adding hydrochloric acid (HCl), this method works because the solution starts at a pH above the pI value. In most of the experiments, the pH value did not correlate with any mechanical or optical behavior. For the latter, TDS can tell one the amount of solvent still inside of the solution after dialysis, but also fibroin protein. Again, this value tended to not help with the understanding of the results regarding mechanical and optical properties, but for other applications in the medical field like drug delivery, these values could be important to understanding the chemical and biological mechanisms at play. In general, the age of the fibroin solution as well as the concentration affected the mechanical and optical properties from fabricating optical thin films.

2.2 Silk Fibroin Films

2.21 Film Fabrication and Thickness of Films

The method predominantly used to form silk fibroin films is a facile casting method. Namely, one should prepare the appropriate substrate and drop the silk fibroin solution on top of the substrate and dry it. If the adhesion of the fibroin solution is weak to the substrate, like on a hydrophobic surface of Polydimethylsiloxane (PDMS), free-standing films are made. Moreover, the films will sometimes detach from the hydrophobic surface after drying, providing easy removal. If the solution is cast on a hydrophilic surface like a glass microscope slide, then a

surface can be coated with a thin film of silk fibroin. These properties will become important when adding nanopatterns to the fibroin films.

The thickness of the films formed depends on the volume of the solution used as well as the volume of the space being filled, and the concentration. Clearly, the less volume of fibroin solution, the thinner the film formed, which is true for a larger surface area as well. Although there seems to be an upper limit for film thickness with a fixed concentration due to how the fibroin dries in a hole mold. No matter how deep the hole is for the fibroin solution, the film thickness will not change that much because instead of the film forming on the bottom surface of the hole, the fibroin solution attached to the walls of the mold will dry and form a film on the walls. For the concentration, it was found that lower concentrations of fibroin solution will produce thinner film and alternatively for higher concentrations producing thicker films. Obtaining a larger thickness was not ideal for these experiments, because the thicker the film becomes, the stiffness also increases. Since fibroin thin films made by the standard method are generally brittle without post-treatment, lower concentration and volume of fibroin solution were used to produce flexible thin films. For this project, most of the fibroin films' thickness ranged from 20 μm to 100 μm not including thick edges due to solution drying on the PDMS mold walls.

2.22 Post-treatment of Fibroin Thin Films

Silk fibroin films made using the standard procedure described previously are water soluble and brittle. The film's stiffness increases as thickness increases. Thus, thinner films are more suitable for new optical applications which are aimed towards flexible devices, but unfortunately are still very weak to tensile stress. There exist a couple of methods to improve the film's properties, either by chemical treatment or humidity treatment.

Chemical treatment involves using certain chemicals that will promote β -sheet crystal structure which can vastly improve the mechanical properties from their original form. Most procedures utilize an 80% methanol or ethanol bath and soak the thin film in the bath for about

1 h. After treatment by methanol, films are softer and more flexible and stretchable. When attempting chemical treatment with ethanol however, the results did not match with other groups' results and this may be due to the results from other groups not accounting for other factors of their environment such as humidity. After chemical treatment, it renders the thin film insoluble to water which is advantageous for applications where the film is exposed to an aqueous environment and should not degrade. One thing to point out about this treatment is that after a few days, the film will revert to its original mechanical properties but will remain insoluble in water.

For the humidity treatment known as the water annealing method, silk films are first prepared by drying in room temperature exposed to the air. Then, the dry film is placed into a vacuum desiccator filled with water in the bottom and the pump is turned on to extract the air out. This exposes the film to an extremely high humidity environment and promotes β -sheet crystal structure like chemical treatment. The film is kept like this for at least one day. Upon removing the film from the vacuum desiccator chamber, the film will be extremely soft, flexible, and stretchable. Again, this result is the same as the chemical treatment. This method is indeed advantageous because it is free of harmful chemicals and vapors like methanol, but there is one major drawback found in this work. Due to the very low humidity in Las Vegas (10-20% RH depending on the day), the film will eventually lose its improved mechanical properties faster than the chemical treatment. The rate is on the order of 3-5 min compared to the several days it takes for the chemical treatment.

The reason for the film losing its mechanical properties in the lower humidity is due to the biopolymer's hygroscopic property. Most of the research done on regenerated silk fibroin protein materials are performed in much higher humidity environments, such as humid regions of China or cities like Boston, Massachusetts where the relative humidity is over 50%. This means that the film will not lose as much water as it does in a lab in Las Vegas. So, the films fabricated will have a more flexible and softer property to them compared to the hard and brittle

films fabricated for this project. Overall this provided a major problem in understanding the properties of the fibroin films and achieving consistent mechanical properties that were desired for a biomedical application, such as flexible and stretchable.

Chapter 3: Fabricating Nanostructured Silk Films

3.1 Soft Lithography Method

Soft lithography is a widely popular technique in nanofabrication for many different materials and technologies and is suitable with a solution of silk fibroin because of the protein's ability to self-assemble. It's a facile method, with no use of harsh solvents or vapors to deposit films and only requires a simple casting procedure much akin to fabricating a simple thin film. If one wants to pattern a film of silk fibroin using this method, it requires three main steps. First, one should choose the appropriate micro or nanopattern they would like to have replicated onto the silk fibroin film and cut it to the size of the polystyrene dish that is to be used. Next, the PDMS mold should be created with the structure replicated onto it with the desired size and depth of the mold. Finally, the fibroin solution should be cast onto the PDMS mold, dried, and simply removed by slowly pulling off with tweezers.

3.11 Experimental Procedure

Choosing the correct structure, feature size, and period for the pattern depends on the application the film will be used in. If one would prefer to use the film as a diffuser for certain wavelengths, the period for the structure should be chosen to the intended wavelength(s). For this work, two different structures were replicated from diffraction gratings. One diffraction grating is a linear structure of 1,000 lines/mm, and one a mesh structure of 13,500 lines/in. Scanning electron microscope (SEM) was used to characterize the surface features. Using the soft lithography method, features of sub-micron scale can be replicated with high quality. Burned DVDs, or etched silicon slabs can also be replicated quite easily by this method to achieve nanoscale structures.

To create the PDMS mold, one should note the size of the mold one is to create. The size of the films created were around 2 x 2 cm using a 35 mm polystyrene dish. For this size,

only about 5 or 10 g of PDMS solution is required depending on how deep one wants the mold to be. PDMS base should be mixed at a 10:1 ratio with the curing agent. When combined, the solution should be well mixed until the bubbles generated are small and difficult to discern. Then, the PDMS solution should be set into a vacuum chamber with the pump on for about 15 min to evacuate the tiny bubbles. This process should be repeated until no bubbles are present in the solution. Once the solution is prepared, the diffraction grating to be replicated should be cut to the 2 x 2 cm size. If the mold is to have a depth greater than the thickness of the diffraction grating, a stack of glass slide covers or any other small block can be placed in the bottom of the petri dish, then the diffraction grating on top, with the structure facing to the top. Then, PDMS solution can be cast onto the grating slowly as to not generate any air bubbles. If using glass slide covers to create depth in the mold, some air between the covers will be forced out by the PDMS solution and will float to the surface. Any additional small bubbles can be evacuated again by the vacuum chamber. The dishes can then be set in an oven to cure at 80°C for 3 hours until the PDMS is stiff. Then, the dish can be removed from the oven, and using tweezers, the mold can be removed from the dish carefully. The diffraction grating or stack of slide covers can be removed gently from the PDMS, then the underlying diffraction grating. To check if the pattern is replicated, one should notice a rainbow color over the area where the diffraction grating was covering.

Finally, to create the patterned silk film, the desired volume of solution to use should be determined. This depends on the required thickness for the film. For the 2 x 2 cm mold, about 0.4 mL of 5 wt% silk fibroin solution worked well. The solution should be extracted into a transfer pipette or syringe and then cast into the PDMS mold. Using tweezers or by tilting the mold, the solution should be spread around the mold to fully cover it. Alternatively, if uniformity in the thickness is a design requirement, spin coating can be used as well when dispensing the solution, but at a very slow speed. If bubbles do appear in the solution, a small transfer pipette can be used to remove them. The solution should be left in the mold with no cover at room

temperature for several hours until fully dried, usually about 3-4 hours was enough. When the film has formed, tweezers can be used to carefully remove the film from the mold and a quick check for the rainbow color over the area of the film can confirm if the fibroin did replicate the structure. If insolubility in the film is required, then the film can be placed into the vacuum desiccator and water annealing can be performed on the film.

3.12 Results and Discussion

Figure 1 show the SEM images of the linear and the mesh diffraction grating masters used for soft lithography.

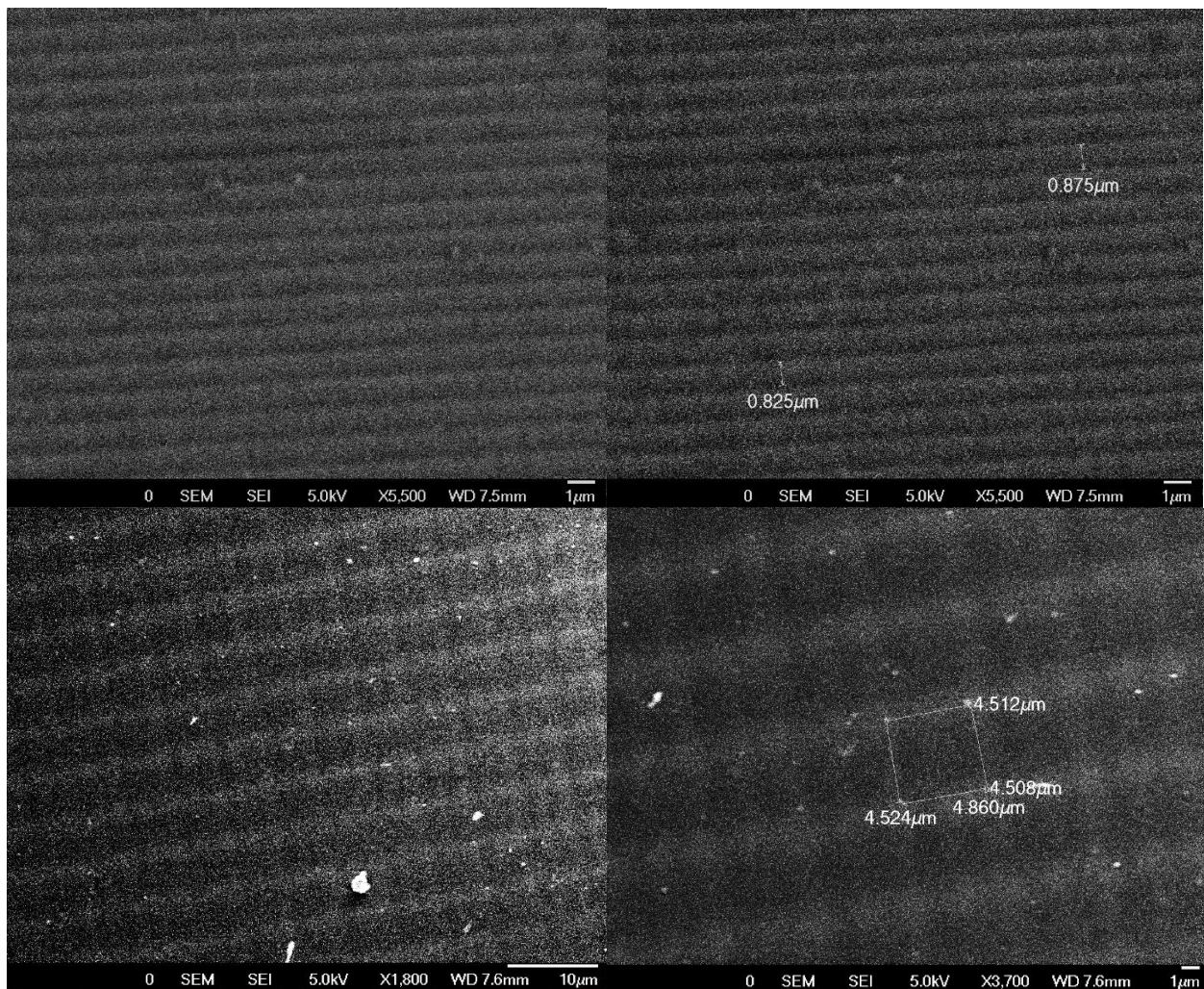


Figure 1: SEM Images of the Linear (top right and left) and Mesh (bottom right and left) diffraction gratings enhanced with ImageJ

Due to the resolution of the SEM machine used, ImageJ was used to enhance the images to be easier to see. The linear structure is a series of lines that are all parallel which can be seen from the top left photo in **Figure 1**. Here, the period size of the lines in the linear structure is about 825 nm. The mesh structure in the bottom left and right photo of **Figure 1** is a set of perpendicular lines that is a periodic pattern, creating a mesh where rectangular spaces exist in between the lines. Some large dust particles were captured in the images, but the general structure can be seen from the bottom left photo in **Figure 1**. For the mesh structure, the space between lines is about 4500 nm on each side, with one side having a spacing of 4860 nm.

From **Figure 2** the patterned PDMS molds fabricated with linear and mesh structures are seen.

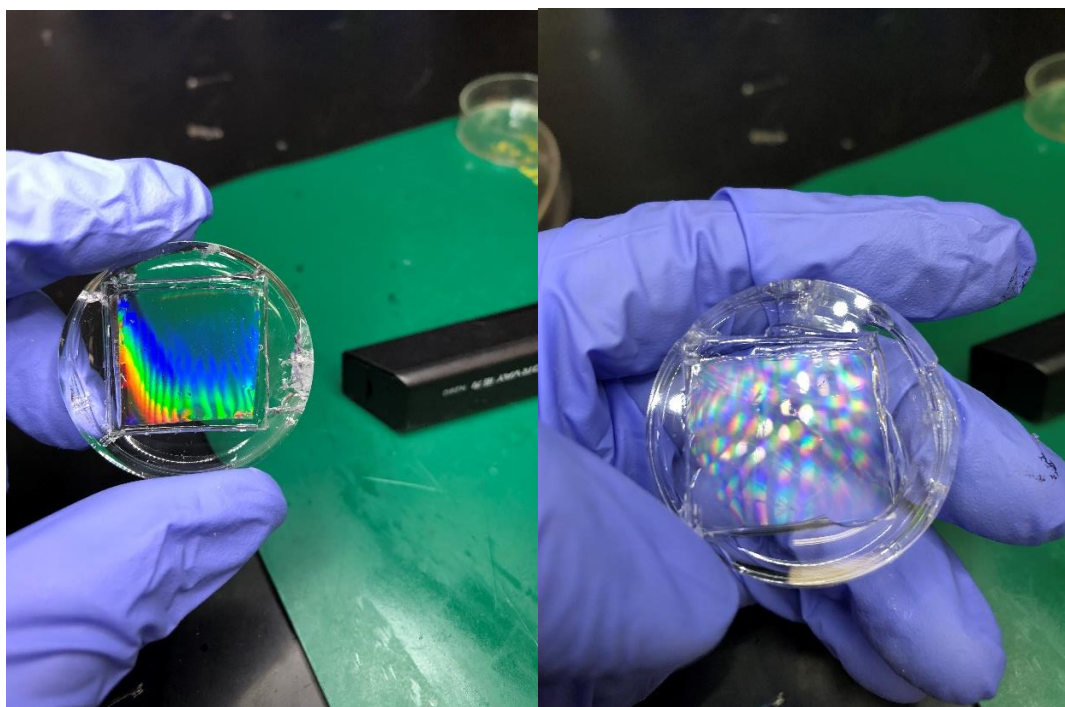


Figure 2: PDMS molds with the linear (left) structure and mesh (right) structure.

Using visual inspection, the rainbow diffracted across the surface shows the pattern was replicated. The optical characterization will also show how light is distributed through the surface similarly as a diffraction grating to further this claim. In the left photo of **Figure 2**, the linear rainbow pattern can be seen, and the haze due to the mesh structure can be seen in the

right in **Figure 2**. Then soft lithography is done with the silk fibroin to replicate the structure from the PDMS mold. The patterned silk thin films after drying are shown in **Figure 3**.

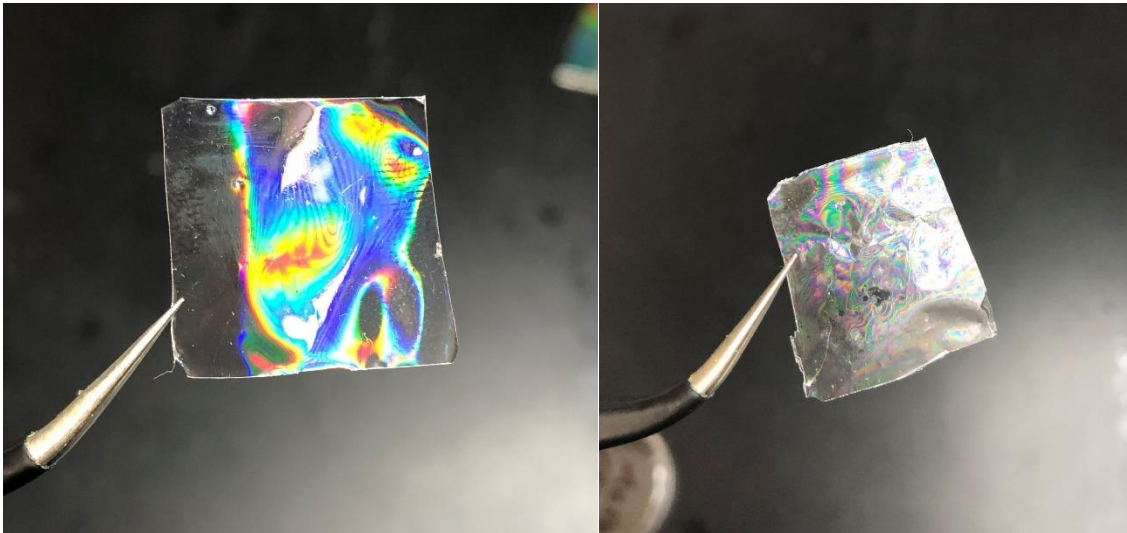


Figure 3: Patterned 1.5 x 1.5 cm 5 wt% silk fibroin films with the linear (left) and mesh (right) structures.

It's more difficult to see the structure in the light for the fibroin film because they are thin and tend to have small folds or curves in the surface. Again, the optical characterization will show that light passing through the films behaves similarly to the PDMS mold, and even the original master of the diffraction grating. The films are cut around their edges to have consistent thickness across the film, so the exact dimensions may not be 1.5 x 1.5 cm.

3.2 Nanoimprinting Method

Another method to pattern silk films that has been receiving new popularity due to its speed and high throughput is the nanoimprint method. Additionally, there is a similar hot embossing method. This follows traditional nanofabrication methods much like the soft lithography method. Again, no harsh solvents or chemicals are necessary to fabricate the patterned films, only water. This water-assisted nanoimprinting method was explored in this work, but issues were found in its repeatability.

3.21 Experimental Procedure

To perform the nanoimprinting method, a solid hydrophilic substrate must be used. This is because the silk fibroin must attach and form a thin film on the substrate. For the experiments, glass microscope slides were used because other groups used the same kind of substrate. However, when building a substrate device, the substrate material will become important. To show the method a glass slide as the substrate material is enough.

First, the microscope slide is placed into an ultrasonic cleaning device filled with DI water for 180 s. Once complete, the slide is taken out and any water is blown off it by compressed air. The substrate is then placed into a plasma cleaner. A Harrick Plasma brand plasma cleaner (PDC-32G) was used as seen in **Figure 4**.

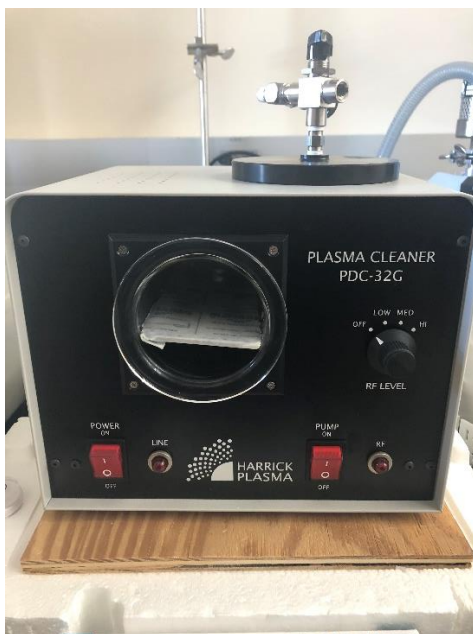


Figure 4: Plasma Cleaner PDC-32G.

The chamber cover is put on and the valve is closed. Then, the device and vacuum pump are turned on and allowed to evacuate the air in the chamber for about 10 min. Next, the radio frequency (RF) level is set to high and the valve is gently opened until only a little air can enter. If done correctly, a violet color is seen, and the plasma is kept on for about 1 min. The RF level

is set to off, the valve is closed, and the pump and device are shut off. The valve is again gently turned until air is allowed in at a low flow rate. Once at equilibrium, the cover is removed, and the substrate is taken out of the plasma cleaner.

Now that the glass slide is prepared, 1 mL of 3 wt% silk fibroin solution is extracted into either a transfer pipette or syringe. The solution is then cast onto the glass slide from the center and can spread across the surface easily. The solution should be left to dry at room temperature for about 3-4 hours.

A PDMS stamp should be prepared. This follows a similar procedure to the soft lithography method of fabricating a PDMS mold. The only difference is that instead of creating depth with something stacked underneath the diffraction grating, no object should be put underneath it. Once the PDMS is removed from the petri dish, the patterned area can be cut out and a rectangular stamp can now be used like in **Figure 5**.

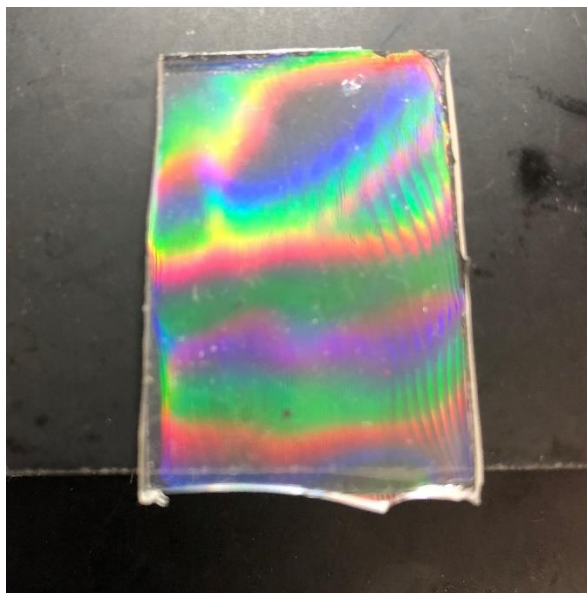


Figure 5: Linear patterned PDMS stamp.

To nanoimprint the silk-coated glass slide, small water droplets should be spread across the patterned surface of the PDMS stamp. About 1 μL of water per droplet is suitable, because bigger droplets will dissolve the fibroin and spread it too much. Then, the silk-coated slide can

be overturned and pressed onto the PDMS stamp with very little pressure, about 30 psi for about 5 min. After, the arrangement should be left for about an hour to allow the water to evaporate. Then, the PDMS master can be removed slowly and the silk-coated slide should have replicated the pattern that can be visually checked by the rainbow colors diffracted in light.

If the film is to be free-standing without the glass substrate, water annealing can be performed on the film to separate it from the glass slide. The film will tend to peel off the slide in increased humidity conditions, and it will also be rendered insoluble. Alternatively, plasma cleaning can separate the silk film from the slide, and this was done on transparent paper substrates as they were harder to separate the silk film by simply water annealing.

3.22 Results and Discussion

Multiple samples were prepared, but none of them had consistent results as far as replication of the master pattern. **Figure 6** is an example of incomplete patterning of a silk film.

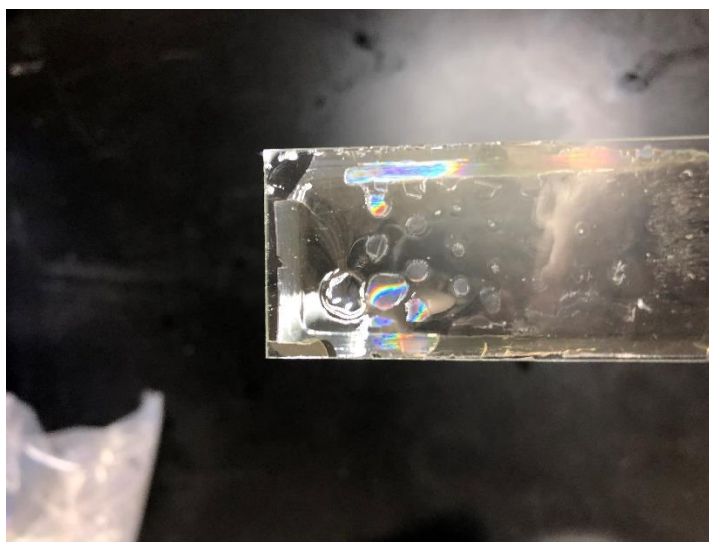


Figure 6: Incomplete patterning of a silk-coated slide.

Here, the small dots with the rainbow colors indicate that the pattern was successfully replicated as well as on the edge where some water had been pushed out. This became a central issue in using the nanoimprint method and brought many of the inconsistencies. The mechanism at play for the nanoimprint to work is to locally modify the relative humidity on the

surface of the film, then the fibroin will reassemble on a pattern being pressed onto it. This works because for saturated silk films, the fibroin has a glass-transition temperature of around room temperature. The key in preserving the fibroin film is controlling its exposure to the water droplets while also being able to apply the optimal pressure to replicate the film. **Figure 7** is an example of too much water being applied to the silk-coated slide.



Figure 7: Nanoimprinted silk film with significant dissolved sections.

Here, the issue was that the fibroin dissolved and did not reassemble with the other portions of the film. So, when the film was water annealed to be removed, some sections were left behind on the glass slide substrate. Even if plasma cleaning were to be used, these sections would have also been separate from the film just the same as it is seen. Notably, the size of water droplets was generally okay, as the film still shows the pattern throughout. The ability to make large-scale patterned films seems to be an underlying issue with this method. For smaller films, the results are more consistent.

Chapter 4: Optical Characterization

4.1 Laser Light Test and Thickness Measurement

4.11 Introduction

This experiment is used to show visually how the light is scattered by the patterned silk film. A 650 nm laser light is passed through the fabricated silk films 10 cm above a target with minor axis tick marks of 5 mm and major axis tick marks of 25 mm to help estimate the hazing effect. Since an atomic force microscope (AFM) was not available for this project, the laser light also helps illuminate how well the pattern was replicated by comparing the scattering from the original pattern master to the patterned silk film. Also, using calipers, the thickness of the films was measured with an uncertainty of ± 0.01 mm.

4.12 Results and Discussion

Figure 8 shows the results for samples with no structure, linear structure, and mesh structure for the master, PDMS, and a 5 wt% silk fibroin thin film.

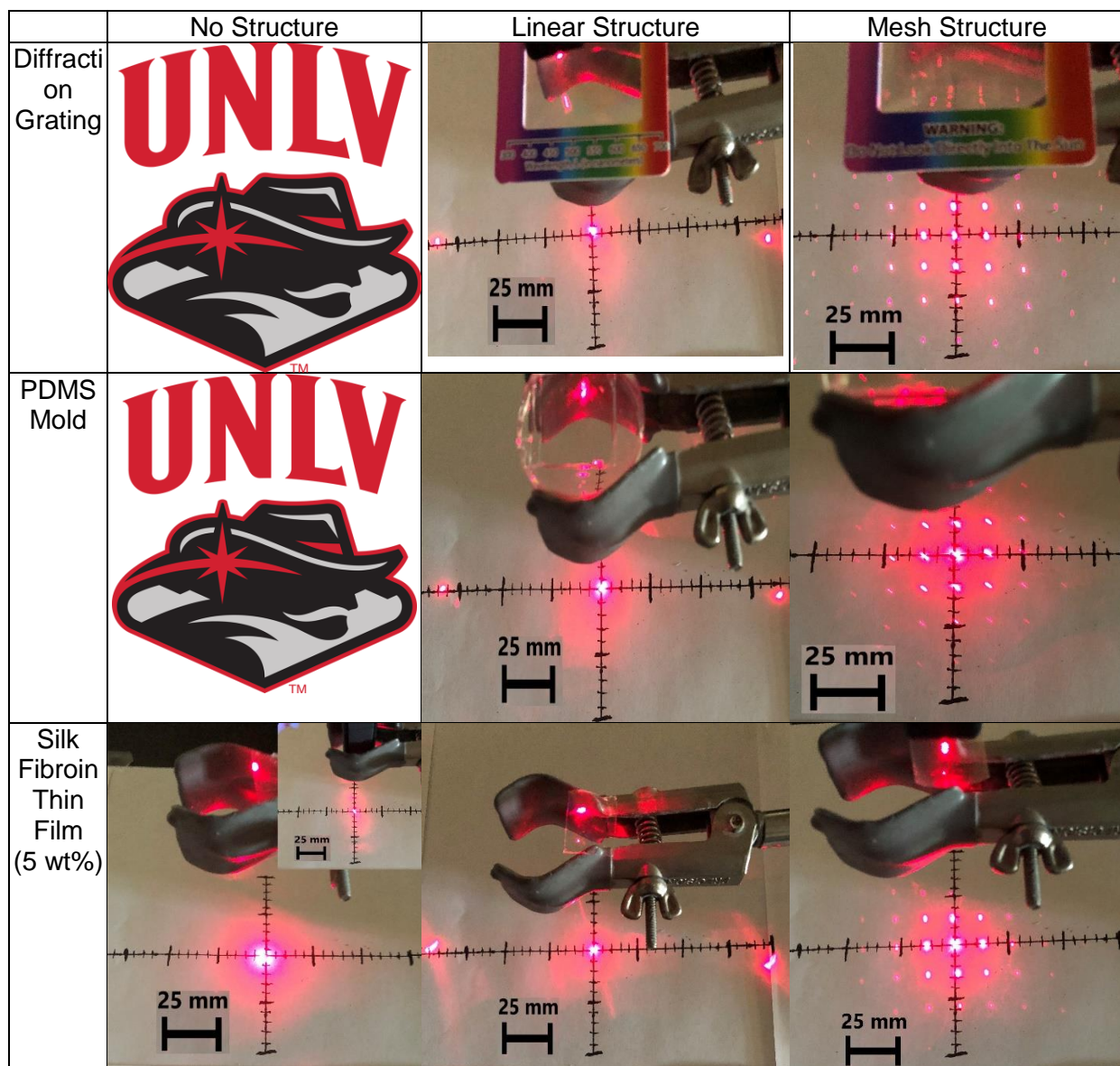


Figure 8: Light scattering of master, PDMS, and films for the laser light test.

From the figure, one can see that as the pattern is replicated from material to material, there is a change in the transmission haze. This can be seen most clearly from the mesh diffraction grating, where initially all the laser light is scattered into many distinct points, with the intensity being distributed evenly across a 2.5 x 2.5 cm square. When replicating to the PDMS mold, the mesh pattern becomes more focused in intensity at the center but there is a hazy red cloud surrounding each point. Finally, on the film the laser light scattering in the silk fibroin film displays haze as well and is comparable to the PDMS mold. Also, in the center point created by

the laser light in the linear structured silk fibroin film, there is a higher haze effect, or a cloud of red light surrounding it compared to the master where the light is distributed into three distinct circular points. This is consistent with the laser light through the film with no structure, which displays a hazy circular cloud surrounding the point of about 25 mm radius as it is transmitted through the film. With the laser point by itself seen in the corner of the bottom left photo of **Figure 8**, it can be seen the light is within a circle of a 5 mm radius. The film does well to replicate the pattern by having the same behavior of scattering the light, unfortunately the study is limited by not being able to study the exact topography of the structure on the film.

For the thickness of the film, since the patterned sections cut out of the molds were not always perfectly level and sometimes the edges of the films would be quite thick due to how the fibroin dried, there was some variance in the measurements. **Table 1** summarizes the measurements of the nine samples analyzed.

N	Average Thickness (μm)	Standard Deviation	Standard Error
9	27 ± 10	8.7	2.9

Table 1: Thickness Measurements of Fibroin Films.

There is a dependence on the thickness with regards to the optical properties, such as the transmission and transmission haze. At the order of 10 micron or less, the impact seemed to be insignificant in the transmission. Although, when the thickness increases by an order of magnitude, the optical properties can be affected significantly, but this was not a part of the scope of the study.

4.2 Spectrometer Analysis

4.21 Introduction

A spectrometer analysis device (Ocean Optics USB4000) was used to investigate the transmission of the fabricated silk fibroin thin films. A light source was connected by an optical

fiber that serves as the input white light and is attached to a collimating lens fixed to a mount pointing downward which serves as the incident light. Another optical fiber is connected to the spectrometer analyzer and is attached to another collimating lens facing upwards and will receive the transmitted light. A small platform for the film is placed on the mount above the receiving optical fiber. The apparatus can be seen in **Figure 9**.

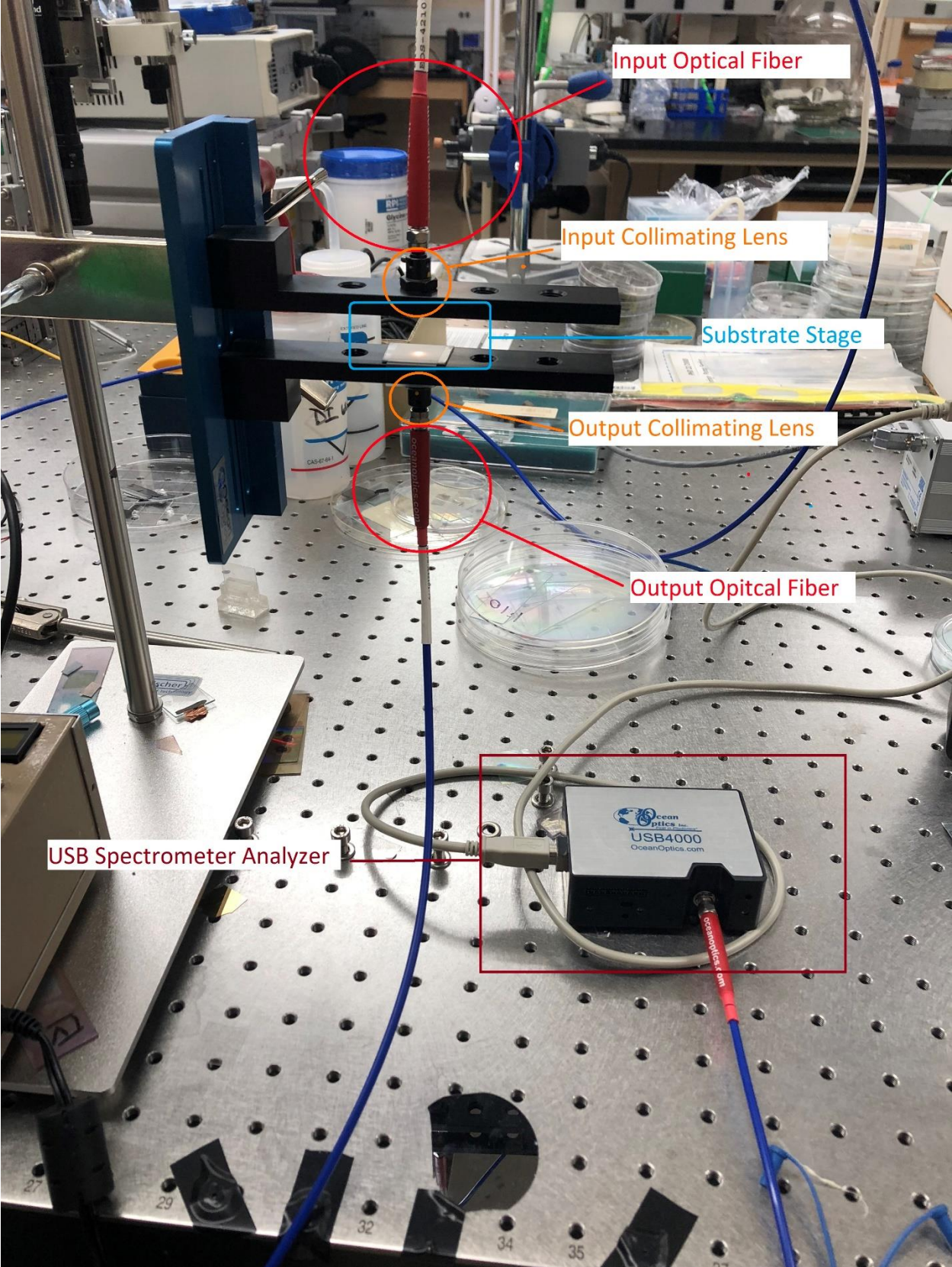


Figure 9: Spectrometer experiment set-up.

The spectra analyzer is connected by USB to a computer with OceanView downloaded as the spectrum analysis software. This apparatus can analyze transmission as well as absorbance and color.

4.22 Results and Discussion

Figure 10 shows the transmission data for the thin film samples as well as the PDMS molds for comparison. The transmission is given over a range of wavelengths but the data for this table was constructed by choosing the transmission measurement starting at 500 nm, which is about the limit of the spectrometer, and incrementing by 50 nm up to 850 nm.

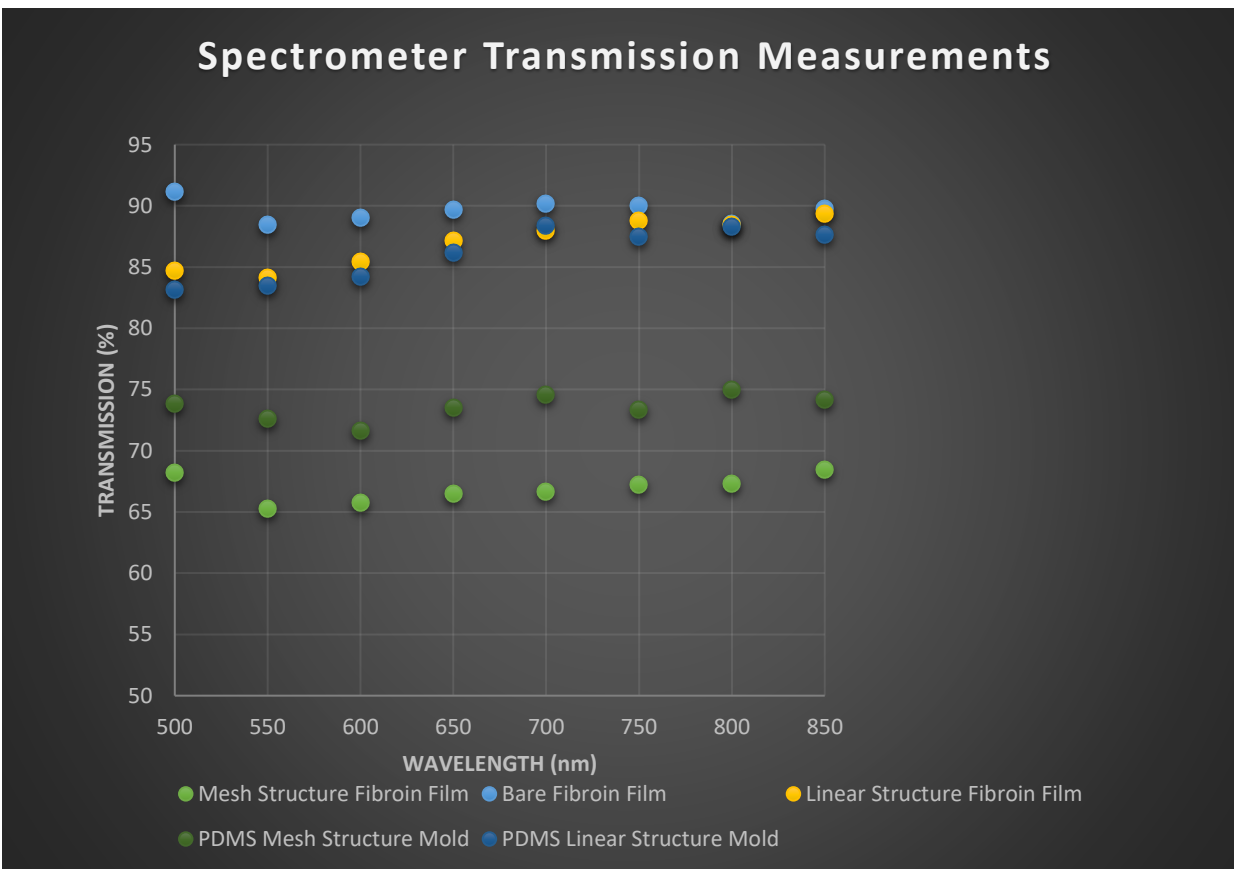


Figure 10: Spectrometer transmission measurements.

Based on these measurements one can notice that PDMS and silk fibroin thin films possess similar transmission, and the addition of structures also has a negative impact on the transmission. With the spectrometer apparatus used, the optical fiber cannot capture light that

is beyond the diameter of the fiber, thus a significant loss in transmission for the mesh structure is seen. This is because there is a larger distribution area of the light, while with a linear structure, the light is only scattered across three points. The more structures that are present, the more refraction and total reflection that is occurring over the surface, thus less light is received on the other end of the light source. Silk fibroin boasts high transmission without any structure, near 90% for most wavelengths. Which shows the real strength of this material in optical applications. Notably, silk fibroin can make a great biodegradable substitute for a flexible anti-reflection coating on a solar cell due its impressive transparency. Plus, with the ability to add patterns, the light scattering is highly improved. This can increase the path length of light traveling through an absorber layer to improve the efficiency of the solar cell. Further, in biomedical applications such as low-intensity photodynamic therapy, the film's biocompatibility combined with its transparency makes it a great material to distribute light through.

4.3 Spectrophotometer Analysis

4.31 Introduction

The final characterization method was to measure the hazing effect quantitatively. The most common way to do so is using a UV-Vis spectrophotometer with an integrating sphere to measure the transmission haze. How this measurement system works is by first obtaining the baseline spectrum. A light should be shined on a white reference plate and the light scattered by the instrument itself is also measured for calibration purposes. The sample can then be put in front of the incident light before it enters the integrating sphere so that the total light transmittance and the sample light diffusion can be measured. The haze is then calculated by

Eq. 2

$$\text{Transmission Haze} = \frac{T_d}{T_t}$$

Eq. 2: Transmission Haze Calculation

Where T_d is the diffused transmission light, and T_t is the total transmitted light. Three groups of each structure were measured including a thin film with no structure. A quartz glass sample was used as a reference substrate.

4.32 Results and Discussion

In **Figure 11**, the results for the sample transmission and transmission haze can be seen.

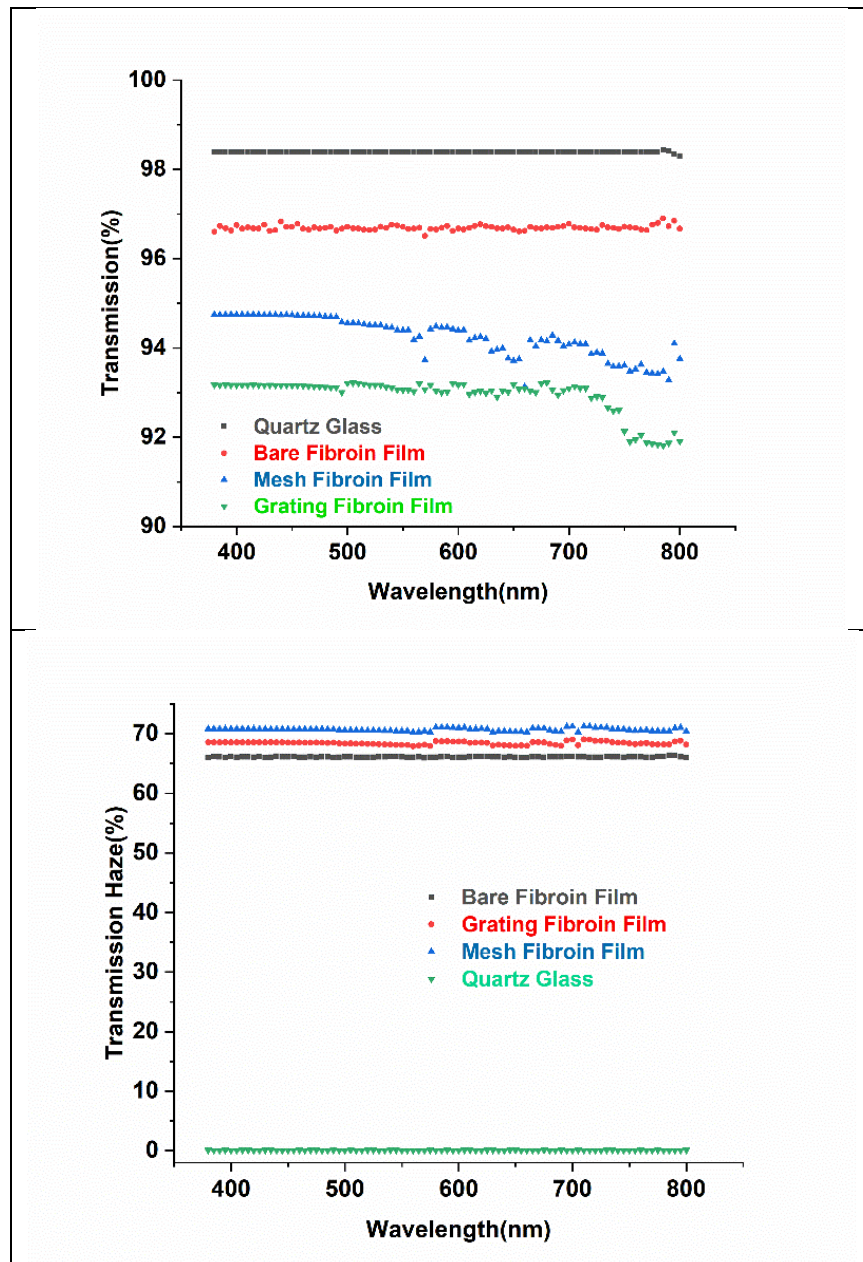


Figure 11: Spectrophotometer transmission (top) and transmission haze (bottom) measurement.

From the transmission data, one can see that all samples produced transmission of over 90% across the wavelengths tested. The reason for the discrepancy in this data versus the spectrometer data may be due to difference in the apparatus. Spectrophotometers use an integrating sphere that can measure all refracted light and give the total transmission. The spectrometer can give a transmittance measurement, but as discussed previously, there is a loss in the transmittance when light is refracted rather than transmitted directly to the output optical fiber. Thus, it's found that spectrophotometers can give a much clearer picture of the transmission for high haze films.

The transmission haze is the real astounding result for this set of data. All the films maintain a high transmission of over 90% and all have a hazing effect of 65% and over with the fibroin film with no structure producing the least transmission haze. It seems that due to the secondary β -sheet crystal structure, the films can maintain their high transparency as well as scatter much more light. Traditional polymers and materials used in optics have found that there is a trade-off for transmission and haze, but with the high stacking density of the self-assembled fiber sheets the transmission haze can be drastically improved without sacrificing transmission light. More light is indeed scattered but there is not a significant increase in the absorption or reflectance. This makes this material form very attractive for applications on solar cells and PDT. For a solar application, the diffusion of the light across a larger area serves to increase the photoactivated surface area and thus increase electron and hole pair production, resulting in a larger external quantum efficiency (EQE) as well as a high power conversion efficiency (PCE). The increased light scattering also increases the path length of light, increasing the amount of absorbed incident radiation which also serves to improve the PCE even further. For a PDT application, the high haze is beneficial when using the fibroin film as a light diffuser, where less intense light can be scattered across a larger surface area for treatment without sacrificing as much intensity as other materials. In other diffusers, higher

intensity light would be used because after passing through the material, there would be a down-shifting effect lowering the intensity of the light, but with the fibroin film this would not be a significant issue.

Chapter 5: Conclusion

Using existing fabrication techniques, the transmission and transmission haze has been investigated for patterned silk fibroin thin films. Showcasing the optical capability of this technology opens new paths for research regarding this protein and new applications that have previously not been considered. Optical sensors, solar cells, and light therapy are major targets for this sort of biopolymer, as it holds great potential in revolutionizing these fields. Eliminating materials that are harmful to the environment, or materials that are expensive to fabricate or synthesize is a major benefit to applying silk fibroin materials. It's a smarter material option for the environment and is applicable to many optical applications due to its good properties in optics and mechanical properties when treated correctly.

This means this study's scope was limited to measurement of a few parameters and variables. β -sheet content of the film was not a focus, nor was the optical density of the crystal structure, but these have an influence on the resultant transmission and transmission haze. The difficulty in using scanning electron microscope (SEM) to investigate the inner crystal structure is that the thin films were so thin that sputter coating the cross-section is a challenge. Other techniques exist but were not readily available to study the optical density. Also, new methods in fabricating silk fibroin solution exist like using formic acid to dissolve fibroin. When using this method, the mechanical properties are less dependent on the ambient humidity such as in this work, and the film is directly insoluble. However, if a dissolvable film is to be used, like in wound dressing, this method will not alleviate the environmental factors on the mechanical properties, but it has many uses.

Other ways to modify and perhaps improve the properties of the silk fibroin materials that could be looked at are in physical or chemical cross-linking. Some works have investigated using other biopolymers such as gelatin and chitosan to cross-link with the fibroin to improve flexibility, but most have ignored the optical properties. Preliminary experiments were

performed on this for this project, but none yielded consistent results regarding transmission haze, but there were changes regarding the optical properties that could be examined more closely. For example, combining the formic acid method as well as physical cross-linking of the fibroin with other proteins like gelatin could yield further improved haze and mechanical properties. The key for a future project on this topic should concern itself with the ratio of proteins in the solution, and the degree of cross-linking between them. Theoretically, higher cross-linking would yield a higher optical density or at least better haze as more light could be scattered by the linked protein molecules. Plus, the robustness could be improved by more favorable properties from proteins such as gelatin or a shape memory property from chitosan.

Finally, based on silk fibroin's light guiding properties, new technologies could be designed and tested. For instance, degummed silk fibers or a silk fibroin hydrogel could be used in combination with a patterned silk film, or a hazy silk film. It is easy to attach a fiber to the drying solution if the correct apparatus is arranged to hold the fiber in place as the solution dries. Then, using an microlensed optical fiber, light can be guided through the fiber into the film to distribute the light throughout the film and onto whatever surrounding surface. The application for this could be for topical nonlinear low intensity PDT, or low intensity PDT on a superficial bacterial infection or tumor. The advantage would be the biodegradable and biocompatible nature of the film, and its ability to be dissolved in the body safely. Further ideas of this technology can include bioluminescent proteins to eliminate the need of a degummed fiber waveguide or hydrogel waveguide. This sort of technology could be implanted in the body, and harmlessly deteriorate while delivering a low intensity light in sections it's needed without majorly invasive surgery or use of high intensity lasers to kill bacteria.

References

- [1] C. Jiang *et al.*, “Mechanical properties of robust ultrathin silk fibroin films,” *Adv. Funct. Mater.*, vol. 17, no. 13, pp. 2229–2237, 2007.
- [2] B. D. Lawrence, F. Omenetto, K. Chui, and D. L. Kaplan, “Processing methods to control silk fibroin film biomaterial features,” *J. Mater. Sci.*, vol. 43, no. 21, pp. 6967–6985, 2008.
- [3] I. Georgakoudi *et al.*, “Intrinsic fluorescence changes associated with the conformational state of silk fibroin in biomaterial matrices,” *Optics Express*, vol. 15, no. 3, p. 1043, 2007.
- [4] S. Sofia, M. B. McCarthy, G. Gronowicz, and D. L. Kaplan, “Functionalized silk-based biomaterials for bone formation,” *J. Biomed. Mater. Res.*, vol. 54, no. 1, pp. 139–148, 2001.
- [5] S. Hofmann *et al.*, “Silk fibroin as an organic polymer for controlled drug delivery,” *J. Control. Release*, vol. 111, no. 1–2, pp. 219–227, Mar. 2006.
- [6] M. Xie *et al.*, “An implantable and controlled drug-release silk fibroin nanofibrous matrix to advance the treatment of solid tumour cancers,” *Biomaterials*, vol. 103, pp. 33–43, Oct. 2016.
- [7] M. Choi, D. Choi, and J. Hong, “Multilayered Controlled Drug Release Silk Fibroin Nanofilm by Manipulating Secondary Structure,” *Biomacromolecules*, vol. 19, no. 7, pp. 3096–3103, Jul. 2018.
- [8] X. Wang, J. A. Kluge, G. G. Leisk, and D. L. Kaplan, “Sonication-induced gelation of silk fibroin for cell encapsulation,” *Biomaterials*, vol. 29, no. 8, pp. 1054–64, Mar. 2008.
- [9] T. Yucel, P. Cebe, and D. L. Kaplan, “Vortex-Induced Injectable Silk Fibroin Hydrogels,” *Biophys. J.*, vol. 97, no. 7, pp. 2044–2050, Oct. 2009.
- [10] D. Su, M. Yao, J. Liu, Y. Zhong, X. Chen, and Z. Shao, “Enhancing Mechanical Properties of Silk Fibroin Hydrogel through Restricting the Growth of β -Sheet Domains,” *ACS Appl. Mater. Interfaces*, vol. 9, no. 20, pp. 17489–17498, May 2017.

- [11] S. Das *et al.*, “Bioprintable, cell-laden silk fibroin–gelatin hydrogel supporting multilineage differentiation of stem cells for fabrication of three-dimensional tissue constructs,” *Acta Biomater.*, vol. 11, pp. 233–246, Jan. 2015.
- [12] B. Zhu *et al.*, “Silk Fibroin for Flexible Electronic Devices,” *Adv. Mater.*, vol. 28, no. 22, pp. 4250–4265, 2016.
- [13] Y. Liu *et al.*, “Highly flexible and lightweight organic solar cells on biocompatible silk fibroin,” *ACS Appl. Mater. Interfaces*, vol. 6, no. 23, pp. 20670–20675, 2014.
- [14] L. Qingsong, Q. Ning, P. Yu, Z. Yafeng, S. Lei, Z. Xiaohua, L. Yuekun, W. Kai, S. K. Ick, “Sub-micron silk fibroin film with high humidity sensibility through color changing,” *RSC Adv.*, vol. 7, pp. 17889–17897, 2017.
- [15] S. Kujala, A. Mannila, L. Karvonen, K. Kieu, and Z. Sun, “Natural Silk as a Photonics Component: a Study on Its Light Guiding and Nonlinear Optical Properties,” *Sci. Rep.*, vol. 6, no. 1, p. 22358, Apr. 2016.
- [16] S. T. Parker *et al.*, “Biocompatible Silk Printed Optical Waveguides,” *Adv. Mater.*, vol. 21, no. 23, pp. 2411–2415, Jun. 2009.
- [17] D. D. N. Rockwood, R. R. C. Preda, T. Yücel, X. Wang, M. L. Lovett, and D. L. Kaplan, “Materials fabrication from *Bombyx mori* silk fibroin,” *Nat. Protoc.*, vol. 6, no. 10, pp. 1–43, 2011.
- [18] Z. Zhou, S. Zhang, Y. Cao, B. Marelli, X. Xia, and T. H. Tao, “Engineering the Future of Silk Materials through Advanced Manufacturing,” *Adv. Mater.*, vol. 30, no. 33, p. 1706983, Aug. 2018.
- [19] M. A. Brenckle, H. Tao, S. Kim, M. Paquette, D. L. Kaplan, and F. G. Omenetto, “Protein-protein nanoimprinting of silk fibroin films,” *Adv. Mater.*, vol. 25, no. 17, pp. 2409–2414, 2013.
- [20] Z. Fang *et al.*, “Novel Nanostructured Paper with Ultrahigh Transparency and Ultrahigh Haze for Solar Cells,” *Nano Lett.*, vol. 14, no. 2, pp. 765–773, Feb. 2014.

Curriculum Vitae

Corey Malinowski

Research Assistant

cbmalinowski@hotmail.com

Professional Summary

As a student at UNLV, I've always tried to challenge myself and take on things outside of the realm of my major in Mechanical Engineering. When moving into a research assistantship, I found myself working in the field of bioengineering, where biology, chemical engineering, and biomaterials were the focus. In this position, I adapted to the challenges of having a traditional mechanical engineer's background, working on proteins and solutions rather than turbines and compressors. I was excited to learn, and I was able to become very knowledgeable on the protein I worked on, silk fibroin, and well-versed on the biological mechanisms affecting proteins. As a professional I grew into a stronger researcher, writer, and scientist, and could clearly express ideas, concerns, and results to my advisors and fellow researchers. I have also worked in the industry as an intern and was recognized as a fast learner and efficient worker. My tasks would always be completed ahead of schedule, and supervisors always had to struggle to keep me busy because of my strong work ethic and high-quality work output. I am always looking to try new things, and I am always welcome to work in fields that I have a lack of experience in so that I can expand my own knowledge and abilities.

Core Qualifications

- Certified Engineering Intern

Professional Qualifications

- A knowledge of 3D modeling software such as Solidworks and Creo
- Experience using COMSOL to evaluate heat transfer problems
- Operation of many laboratory devices like spin coaters, centrifuges, sonicators, and spectrometers
- Ability to use nanofabrication methods such as soft lithography and nanoimprinting
- Chemistry lab experience

Experience

2016-Present: UNLV Research Assistant. Responsibilities included:

- Fabricating protein solutions using various chemical processes like degumming, dissolving, purification by dialysis, and centrifugation
- Fabricating thin films and patterning using nanofabrication methods
- Creating hydrogels by following and modifying published methods
- Modifying hydrogels with antimicrobial silver nanoparticles to create antimicrobial hydrogels
- Keeping up to date on current research, methods and procedures to improve processes used
- Devising a research direction and experimental platform for sample testing
- Optical characterization of samples using qualitative and quantitative methods
- Data management and organization
- Generating reports and presentations to present to advisors

2017-2017: Nikkiso Cryo, Inc. Engineer Intern. Responsibilities included:

- Verifying Bill of Materials (BOMs)
- Addressing customer comments on pump drawings and documents

- Closing legacy projects by following up on document trails
- Data management
- Generating reports for pump data

Education

- University of Nevada, Las Vegas. Bachelor of Science in Engineering – Mechanical Engineering, Fall 2013-Spring 2017

References

Dr. Hui Zhao (Technical Advisor, mentor)

Associate Professor

Mechanical Engineering Department

University of Nevada, Las Vegas

Office: SEB 2174

Phone: 702-895-1463

Email: hui.zhao@unlv.edu

Dr. Shengjie (Patrick) Zhai (Supervisor, Lab manager)

Research Scientist

Mechanical Engineering Department

University of Nevada, Las Vegas

Office: SEB 2215

Phone: 702-744-1409

Email: shengjie.zhai@unlv.edu