Designing a Synthetic Spider Silk Based Coating for Urinary Catheters to Reduce the Risk of CAUTIs

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DESIGNING A SYNTHETIC SPIDER SILK BASED COATING FOR URINARY CATHETERS TO REDUCE THE RISK OF CAUTIs

by

Tess Marie Armbrust

Capstone submitted in partial fulfillment of the requirements for graduation with

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Abstract

Catheter associated urinary tract infections (CAUTIs) are a leading cause of morbidity and mortality in patients with life-threatening conditions. Foley catheters are the most common catheter used when a patient requires hospitalization but can become burdened with microbes which lead to CAUTIs, the most common source of hospital acquired infection (HAI). The attached bacteria can form biofilms on the catheter which are difficult to treat using oral antibiotics and can lead to chronic infections. Synthetic spider silk as a catheter coating shows great promise because of demonstrated strength, biocompatibility, and antimicrobial properties. In addition, the antiseptic chlorhexidine can enhance the antimicrobial properties of synthetic spider silk coatings. This design project focused on creating a synthetic spider silk coated Foley catheter that met design needs dictated by catheter usage in nursing clinical practice guidelines. Foley catheters were coated with 6% w/v synthetic spider silk and 6% synthetic spider silk with chlorhexidine using five different coating protocols (spray, dip, aerosolize, dip and aerosolize, and spray and dip). Catheters were tested for increased antimicrobial properties by utilizing zone of inhibition testing. Mechanical testing included force of insertion measurements recorded on an MTS and a bend/bunch test created by the design team. SEM and AFM images were also taken in order to characterize the different coating methods. The final product introduces a novel approach to catheter coatings in an attempt to combat the threat of CAUTIs.
Acknowledgements

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Project Summary
Urinary and Intravenous (IV) catheters are two of the most commonly used medical devices for administering vital drugs and obtaining diagnostic samples from patients. Unfortunately, hospital acquired infections (HAIs) occur at alarming rates due to catheter usage. The aim of this project was to design a urinary catheter that would decrease the occurrence of these infections with a catheter coating that utilizes the antimicrobial properties of synthetic spider silk in combination with the antiseptic chlorhexidine. This synthetic spider silk catheter will introduce a novel approach to catheterization in the medical industry.

1. Introduction
Intravenous catheters have been used for almost 350 years as an integral tool in patient care. IV catheters are used in 60-90% of patients admitted to hospitals each year and introduce a potential source of infection (Helm, 2015). Annually, 16,000 central-line associated infections occur in intensive care units around the United States, with approximately 500 to 4000 deaths from catheter related bloodstream infections (Mermel, 2000). These infections are caused by the formation of microbe colonies on the catheter and the skin surrounding the insertion site.

Advances in the catheter industry such as the Dacron cuff and antimicrobial coatings decrease the rate of infections caused by catheterization. Most tunneled central venous catheters are equipped with a Dacron cuff to introduce an inflammatory response that keeps the catheter in position. It also acts as a physical barrier to microorganisms that are present on the skin. Additional strides have been made to combat infection by investigating the use of different catheter materials and biocompatible coatings.

Mechanical failure is another catheter problem. Peripheral venous catheters (PVCs) have failure rates between 35-50% that are caused by phlebitis, infiltration, occlusion, and dislodgement (Helm, 2013). Phlebitis is inflammation of a vein caused by trauma to the walls of the vessel and is combatted by smaller-gauge catheters. Infiltration occurs when the vein is perforated by the catheter and leads to the inadvertent administration of medications into tissue. Occlusion is a partial or complete blockage in the catheter and can delay the administration of medications. Dislodgement can be a result of movement and leads to other failure in the body. These problems are addressed using different catheter gauges and mechanical improvements. The composition and surface characteristics of catheters also contribute to failure. To alleviate this problem, catheters have been created out of softer, smoother, and less porous plastics such as polyurethane.

The most common urinary catheter in use is the Foley catheter, which has similar drawbacks that lead to catheter-associated urinary tract infection (CAUTI). CAUTIs are the most common hospital acquired infections (HAIs) and are a leading cause of morbidity and mortality in patients with life-threatening conditions. The incidence of CAUTI is more common in females and it increases with age and frequent disconnections. Infections occur from bacterial colonies found on skin and are introduced into the body when the catheter is inserted (Khan, 2016). Bacterial
colonies that cause CAUTIs are typically different from the microbes that cause bloodstream infections from central venous catheters.

Certain material coatings lead to increased antimicrobial activity. It is important to consider the surface properties, antimicrobial activity, and biocompatibility when designing and testing a new catheter, whether IV or urinary. Several different tests have been performed to evaluate surface properties, tensile strength, antimicrobial and biocompatibility properties, and to minimize contact with vessel walls. It is also important to ensure that the catheter can maintain these properties during long term use within the human body.

The initial objectives of this project focused on updating current IV catheter coating methods to implement a coating step in assembly line mass production of IV catheters. Initial mechanical testing and standard operating procedures (SOPs) for coating catheters were created. Upon further examination of coating methods and assembly line production of IV catheters, the project objectives were reevaluated. New objectives focused on coating Foley catheters using three IV catheter coating methods adapted to coat Foley catheters. These methods have been evaluated for their roughness, friction coefficients, and antimicrobial properties. The coatings were further evaluated for their ability to stick to the catheter when the catheter is bent in a manner to simulate Foley catheter usage.

a. Significance and Innovation
Catheters have medical applications that range from peripherally inserted central venous catheters (PICCs), to suprapubic and indwelling urinary catheters. PICCs have a fail rate that ranges between 30–50%. The three most common problems that lead to their inability to be maintained throughout the entire therapy of a patient are infections, fibrin deposition, and thrombotic occlusion. There is research being performed to combat these fail types individually, but not simultaneously. The fail time for PICCs inserted in a non-central vein is approximately 11.4 days and central veins take 16.6 days before failing (Thiagarajan, 1998). As the time before failing increases, a direct negative correlation to the fail rate can be expected.

The antimicrobial properties of synthetic spider silk are widely reported. Other antimicrobial coatings have been used to decrease the rates of HAIs due to catheter usage, however, no coatings have utilized synthetic spider silk. This project will create novel catheters for urinary use that have enhanced antimicrobial properties due to synthetic spider silk coatings. A standardized protocol for various coating techniques will be developed that will allow for uniform and consistent coating thicknesses.

As mentioned above, the original goal of the project was to modify existing intravenous catheter coating methods to methods for mass production of synthetic spider silk coated catheters (Lewis, 2016). Initial testing was performed and further research showed existing methods to be compatible with catheter production lines. Therefore, due to the lack of a design element in the original project objectives, the decision was made to coat Foley catheters, in the hopes of reducing CAUTI rates.
2. Project Objectives
The aim of this project was to create a synthetic spider silk coated Foley catheter that met design needs dictated by catheter usage in nursing clinical practice guidelines. Urinary catheters must be inserted through the urethra, smoothly snake up through the urethra and into the bladder. The synthetic spider silk coated Foley catheters must have a roughness comparable or better than current Foley catheters. They must also exhibit a lower or equal insertion force. The synthetic spider silk coating must provide an antimicrobial effect greater than that of uncoated Foley catheters. Finally, the adhesion of the coating to the Foley catheters must be strong enough to avoid flaking during a bend and bunch test developed by the team.

3. Evaluation Criteria
- Requirements
  - Measured qualitatively using Atomic Force Microscopy and Scanning Electron Microscopy
  - Exhibits either a smoother surface or equally smooth surface to uncoated catheters
- Insertion Force Requirements
  - Measured using MTS Instron
  - Requires statistically less insertion force or insertion force equal to uncoated catheters
- Antimicrobial Requirements
  - Coated catheters exhibit statistically significant larger zones of bacterial inhibition (using three CAUTI-causing bacteria).
- Coating Adhesion Requirements during Bending/ Bunching
  - When bent at various angles and when bunched, catheter coatings may crack, but cannot flake off the catheter.

a. Design Context
A zone of inhibition test was utilized to test the antimicrobial properties of the final catheter coatings. Antibiotic-resistant strains of bacteria, particularly methicillin-resistant \emph{Staphylococcus aureus} (MRSA) have become a more prevalent issue in the medical field and were included in the tests. It was proposed that the Kirby-Bauer method be used to measure the resistance of the bacteria to the antimicrobial coating. An agar plate was uniformly streaked with the bacterium to be tested; then, small pieces of coated catheters were placed on the surface. As the antimicrobial diffused from the surface of the catheter, a zone around the catheter material appeared where no growth occurred, also known as the zone of inhibition (Brown, 2015).

In addition to antimicrobial testing, a friction test method was also designed to accurately measure the force of insertion of the coated and uncoated Foley catheters based on surface properties. Catheters were attached to the load cell of a vertical MTS machine and inserted through a layer of silicone material, similar in durometer to human skin. This system was able to measure the difference in forces caused by various coatings in comparison to an uncoated Foley catheter.
SEM imaging was used to measure the thickness and uniformity of the coatings. Imaging also revealed if the coating was attached firmly to the catheter or not. Samples were cut transversely to an approximate thickness of 1 cm, secured to a mount, and placed in the machine for imaging. AFM imaging was used to measure the roughness of the coatings. Catheters sections were cut into strips about 8 cm long, secured to a glass slide using double-stick tape, and placed in the machine for imaging.

Elderly male patients often have enlarged prostates, which can cause Foley catheters to bend and/or bunch when pushed against the prostate. Therefore, a bend and bunch test was applied to coated catheters. This test recorded the ability of the device to conform to predetermined relevant angles or radii. It was expected that the device would maintain acceptable mechanical properties following the administration of the bend and bunch test. A similar torque test could have been applied to measure these qualities as well ("Flexibility Kink Resistance," 2012).

4. Background

a. Anatomy of Catheter Usage

Intravenous catheters are one of the most commonly utilized medical interventions for administering medication directly into the patient’s bloodstream. Intravenous catheters are a plastic holding mechanism with a small needle extruding from them. The needle is surrounded by a thin plastic cannula. After the catheter is inserted into a vein, the needle is removed and discarded, and the plastic cannula is left inside the vein. The cannula is connected to external tubing which can be used to administer medications. This process is shown in Figure 1 (Nucleus Medical Media, 2009).
Proper Technique for the insertion of an Intravenous Catheter

A. Insert the needle of choice bevel up at a 30-40 degree angle.

B. Advance the catheter to enter the vein until blood is seen in the flashback chamber. A steady backflow of blood indicates successful entry.

C. After the catheter tip and bevel are in the vein, advance the catheter forward off the needle and into the vein. Blood may ooze from the catheter indicating successful entry.

D. Dispose of the needle and connect the plastic cap to the catheter. Adjust flow rate as desired.

Figure 1: The correct process for insertion of an intravenous catheter. Note the removal of the needle and the cannula that is left inside the patient's vein.

Human skin is made of three layers: the epidermis, dermis, and hypodermis. The epidermis is the most external and is responsible for protection against harmful radiation from the sun. Below the epidermis is the dermis, which contains nerve endings, sweat glands, hair follicles and blood vessels. The hypodermis is a layer of adipose (fat) tissue below the dermis. The blood vessels inside the dermis are the target for both venipunctures and intravenous catheters. Veins in this layer tend to be more superficial and visible than arteries. Veins and arteries are also distinguishable by palpitation. Veins are bouncy while arteries have a pulse (Netter, 2014).

Urinary catheters are used in many patients to obtain sterile urine samples or relieve full bladders. These catheters are inserted into the urethra and are snaked into the bladder. Once there, urine flows out through the catheter. Foley catheters are catheters placed for long term use and have a balloon that is inflated with saline once inside the bladder to hold the catheter in place. Male and female urinary catheters are different due to anatomical differences. Men have much longer urethras and must have longer catheters. The male prostate can also complicate
catheterization. As men age, their prostates enlarge and cause the urethra to contract. As a result, catheters with a rigid tip, called Coudé catheters, must be used to force a way through the prostate. Figure 2 shows the anatomy of the male prostate. The female urethra is much harder to locate which can cause difficulties in catheter insertion (Netter, 2014). Figure 3 shows the comparison between a female and male urinary catheter (Duyao, 2015).

Figure 2: An enlarged prostate can require the use of a Coudé catheter in male patients.

Figure 3: The differences between a male (left) and female (right) catheter are shown. Note the longer catheter used in the male patient.
b. Current Issues

Hospital acquired infections (HAlS) are a leading concern among the global medical community. Approximately 800,000 patients each year in the United States are introduced to an infection while in hospital care. These infections cost the medical industry an estimated $4 billion annually. Critically ill patients in intensive care units have the greatest risk for contracting an infection due to increased exposure compared to other areas of the hospital and weakened immune systems. The most common HAIs are urinary tract infections (UTIs), pneumonia, and primary bloodstream infections. Approximately 64% of HAIs are caused by gram-positive bacteria, 27% are caused by gram-negative bacteria, and 8% are caused by fungi (Edmond, 1999). Necessary medical devices such as catheters are one of the leading mechanisms for these infections to occur. Many HAIs are a result of drug resistant strains of bacteria such as methicillin-resistant *Staphylococcus aureus* (*S. aureus*) (Edmond, 1999).

Medications are administered to patients in three primary ways: orally, intramuscularly, or intravenously. Medications given orally are absorbed through the digestive system and into the bloodstream, usually by either the stomach (as in the case of nonsteroidal anti-inflammatory drugs) or the small intestine (as in the case of acetaminophen). Though these medications last much longer than medications given through other administration methods, they do not act quickly. Medications such as norepinephrine, which is used to raise blood pressure in cardiac arrest patients, or haloperidol, which is used to sedate violent patients, must act immediately. Intravenous medications such as these are injected directly into the bloodstream, meaning they act much quicker. However, as a result, they do not last nearly as long. Some medications are harmful to the inner linings of veins and cannot be given intravenously. Because of the damage they cause when administered intravenously, they are given intramuscularly. This allows for a faster route to the bloodstream than oral medications, but it doesn’t damage veins (Spencer & Gilliam, 2015; Vacca, 2013).

PVCs are the most common tools used to administer drugs directly to the bloodstream. These are inserted into a small vein, usually located on an extremity, and are used in 60-90% of patients admitted to United States hospitals. Based on the age of the patient, the size of available veins, and the medication that must be given, different sizes of PVCs can be used. 22 or 24 gauge catheters are generally used for pediatric patients. Adult patients receiving medications such as vancomycin require 18 gauge or larger PVCs. PVC failure rates vary between 35-50%, and occur in the form of phlebitis, infiltration, occlusion, dislodgment, and infection (Helm, 2015).

Phlebitis is the inflammation of a vein caused by trauma to the walls of the vessel. There are two types of phlebitis: superficial phlebitis and deep vein thrombophlebitis (DVT). Insertion of PVCs can cause superficial phlebitis which is usually not harmful to the patient. However, they can increase the chances of an HAI, which can cost thousands of dollars in treatment per case. Even in cases where phlebitis does not cause infection, it can require extra treatment, raising the costs of hospital stays (Feldman, 2004). Phlebitis risk factors include inactivity, which explains why most of phlebitis cases occur in intensive care units. These patients are usually bed-ridden for most of their stay. Phlebitis is treated by pain control, elevation, and warm compresses, provided it is discovered early. However, delayed detection of superficial phlebitis can lead to infections,
as discussed above (Tintinalli, 2016). An image of phlebitis is shown in Figure 4 (Smeltzer, 2010).

![Image of Phlebitis](image1.png)

*Figure 4: Phlebitis in a patient. Note the erythema (redness) surrounding the catheter insertion site.*

Infiltration occurs when the PVC is placed improperly or dislodges and perforates the side of the vein. This causes medications given through that catheter to leak into the surrounding tissue. This can lead to tissue damage, infection, necrosis of the tissue, and amputation. When a PVC is inserted, it is flushed with approximately 10 mL saline to ensure that the catheter is inside the vein. If it has infiltrated, the saline will flush into the surrounding tissue, causing a visible bump, and the catheter will be removed. Infiltration can also occur due to patient movement. This can be much harder for a provider to notice until new medication is given (Feldman, 2004). A diagram of infiltration is shown in Figure 5 (Smeltzer, 2010).

![Diagram of Infiltration](image2.png)

*Figure 5: Infiltration of a vein. If medications were administered they would leak into tissue surrounding the vessel.*
As mentioned above, certain medications are harmful to the inner lining of veins. If medications such as those used for chemotherapy leak into surrounding tissue, they can damage the tissue as well as the surrounding blood vessels (Robl, 2016). Infiltration is the inadvertent administration of non-vesicant drugs, or drugs that do not damage the tissue they come into contact with. In contrast, extravasation is the inadvertent administration of vesicant drugs and can lead to tissue necrosis and potential amputation (Feldman, 2004). Risk factors for PVC infiltration include diabetes, hypertension, dehydration, and local trauma due to frequent PVC insertion or IV drug use. Conditions such as diabetes, hypertension, and dehydration cause the walls of veins to thin and therefore lose structural integrity. Local trauma causes scarring and makes it harder for the healthcare provider to insert the PVC correctly and can weaken the walls of veins. Infiltration can be treated by either cold or warm compresses to either limit exposure to tissue or stimulate blood flow to dilute the medication (Kagel, 2004).

Occlusion occurs when the catheter is obstructed in some way. Hard, or complete occlusions, occur when the obstruction completely blocks the pathway of medications. Soft occlusions occur when the pathway for medications is only partially blocked. The former is far more dangerous because they cause an increase in pressure in the catheter line and cause the vein with the PVC to collapse. Furthermore, occlusions can delay the administration of vital medications (Scott, 1996). A diagram showing the difference between hard and soft occlusions is shown in Figure 6 (Genetech, 2017).

![Figure 6: A soft occlusion (left) partially blocks fluid flow while a hard occlusion (right) completely blocks fluid flow.](image)

The main concern with any medical procedure is infection. Hospitals are constantly attempting to lower the rates of HAIs, and PVCs can cause bloodstream infections. HAIs cost hospitals nearly $10 billion annually and they increase the length of stay for patients. Hospital acquired bloodstream infection rates range from 1.3 to 14.5 infections per 1000 hospital admissions. Every year 62,500 people die of these infections (Zimlichman, 2013; Heister, 2017).

In the past, bloodstream infections originated from gram-negative rods. However, the rate of bloodstream infections caused by gram-positive cocci has surpassed the rate of infections caused by gram-negative rods. This is thought to be a result of the increased use of intravenous catheters. For example, at least one third of all S. aureus (gram-positive coccus) infections
caused in a hospital are a result of intravenous catheters. An infection from \textit{S. aureus} can lead to complications such as MRSA, which typically requires 4 to 6 weeks of daily IV therapy (Meier, 1998).

Patients who are paralyzed, temporarily bedridden, or patients who have had epidurals do not have the ability to urinate normally. Depending on the situation, medical professionals use either short-term or indwelling urinary catheters. Concerns with urinary catheter usage include patient sex, type of urinary catheter, blood clotting in and around the catheter, and infection. When using a catheter, a larger lumen diameter catheter is used for male patients, a smaller lumen is used for female patients, and the smallest diameter is used for infants. Because male urethras are much larger than female and infant urethras, catheters used for male patients must have larger lumen diameters (Stephens, 2017).

There are two main types of urinary catheters: quick-caths and Foley catheters. Quick-caths are catheters inserted into the bladder to collect a urine sample or empty a bladder and are immediately removed. The main issue associated with these catheters is insertion trauma, especially in male patients. However, significant long-term effects of quick-caths are minimal (Fink, 2012). Foley catheters come in two styles depending on the intended dwell time. Regular Foley catheters are intended for shorter dwell times while patients requiring catheters for long term use utilize silver-coated urinary catheters. The antimicrobial properties of silver are useful because infection rates increase as dwell time increases (Hashmi, 2003). Guidelines for dwell times requiring silver-coated urinary catheters are facility-dependent.

Foley catheters are far more common and come in various lumen diameters, measured on the French scale. The diameter in millimeters is equivalent to the French measurement divided by 3. However, many elderly male patients have enlarged prostates. This causes resistance in the insertion process. Healthcare workers can use Coude catheters which have a stiff tip to help push the catheter through any inflamed prostate tissue. These types of catheters cause more pain and are therefore used less frequently than Foley catheters (Stephens, 2017).

Another concern with enlarged prostates is the possibility of blood clots forming in and around the catheter. When this happens, catheters must be irrigated and flushed. Blood clots form when blood encounters a surface, such as a catheter, and initiates the intrinsic pathway of the coagulation cascade, forming a clot that occludes the catheter (Ratner, 2013).

The primary concern with any urinary catheter is a urinary tract infection (UTI). 95% of UTIs in critical care patients are a result of urinary catheter usage and are caused by a variety of different organisms (Rupp, 2004). Catheter-associated urinary tract infections (CAUTIs) are one of the most common HAIs that patients receive during their hospital stays (Rupp, 2004). CAUTIs contracted in the first few days of catheter use are caused by \textit{Staphylococcus epidermidis} (\textit{S. epidermidis}), \textit{Enterococcus faecalis}, and \textit{Escherichia coli}. However, as dwell times increase, CAUTIs can also be caused by \textit{Proteus mirabilis} and \textit{Pseudomonas aeruginosa} (Stickler & Sabbuna, 2007).
CAUTIs follow a specific progression. Bacteria attach to the uneven surfaces of catheters which contain microscopic imperfections (Stickler, 2003). Bacteria can attach either intraluminally (inside the catheter) or extraluminally (outside the catheter between the urinary tract and the catheter) (Garcia, 1999; Tambyah, 2004). The bacteria then form an antibiotic resistant biofilm which harbors, nurtures, and protects bacterial growth on the catheter. These biofilms infect the bladder (Kumon et al., 2001; Cormio et al., 2001; Burton et al., 2006; Trautner & Darouiche, 2004; Helm, 2015).

c. Current Antimicrobial Solutions
Antibiotic resistance is a major concern in today’s medical community. Over the course of the past several years, antibiotics have not improved and diseases such as MRSA and other “superbugs” are on the rise. Approximately 70% of infections that happen while a patient is in the hospital are bacterial infections that are resistant to at least one traditional antibiotic used for treatment. They are hard to combat without providing a “cocktail” of antibiotics which leads to antibiotic resistance (Lara, 2009).

The source of most catheter-related bloodstream infections occurs in catheter hubs and the skin surrounding the insertion site. Therefore, most research is focused toward preventing bacterial colonization at these locations to keep the bacteria from spreading into the bloodstream and creating a biofilm on the surface of the catheter.

UTIs are the most common hospital acquired infection in patients who are in intensive care units (ICUs). The infection can travel up the urinary tract and into the kidneys which causes inflammation. If the pathogenic organism reaches the bloodstream, the patient can become septic (infected). Catheters account for more than 40% of cases of sepsis in acute-care hospitals (Samuel, 2004).

i. Metal Coatings
Various metals exhibit antimicrobial properties. Silver is the most popular metal for catheter coating because it combines high antimicrobial activity with low human toxicity (Schierholz, 1998). Since the 19th century, silver compounds have been used in a variety of antibacterial solutions. The properties of silver as a medical compound have been recognized for about 2,000 years (Prabhu, 2012).

Unfortunately, there are drawbacks to this approach. The manufacturing costs of these catheters are very high. A silver alloy-coated catheter costs $13 compared to $7 for a standard catheter (Saint, 1998). Silver can affect patients differently based on gender because men and women develop CAUTIs differently. The catheters are more effective at preventing infection with gram-negative rods (most common infection in women) than gram-positive cocci (most common infection in men). For this reason, women benefit from the silver-coated catheters more than men (Saint, 1998). Metals may also leach poison into the patient’s body. A product under United States patent US5520664A seeks to solve this issue by embedding silver into a catheter to ensure that the atoms of the antimicrobial metal are substantially non-leaching.
ii. Antibiotics and Antiseptics

Antibiotics such as vancomycin have been shown to reduce the risk of catheter-related bloodstream infections. Centers for Disease Control and Prevention guidelines recommend against prophylactic use of vancomycin because it is an independent risk factor for acquisition of vancomycin-resistant enterococci (Mermel, 2000). It has been suggested that additional antibiotics such as minocycline and rifampin be used to avoid systematic use of a single antibiotic. However, antibiotic coatings are more expensive and can lead to antibiotic resistance (Kusek, 2012; Samuel, 2004). Therefore, solutions should be focused on interventions that will not encourage the emergence of such resistance.

Antiseptic coatings also show antimicrobial effectiveness. The cuff of the catheter, known as the Dacron cuff, is an important site for this type of coating because it is tunneled into the patient's skin to provide a tissue-interference barrier. The Dacron cuff is used to introduce an inflammatory response within the tissue. The response acts as a barrier to microorganisms and helps prevent dislodging of the catheter. Short-term use catheters that are coated with antiseptics, such as chlorhexidine and silver sulfadiazine, reduce the risk of central venous infections. Not only do these methods decrease the risk for infection but also reduce the cost per catheter. Antimicrobial activity of the catheter decreases over time so antiseptic coatings should only be used for short-term catheters that are in use for less than two weeks (Mermel, 2000).

iii. Nanoparticles

Nanoparticles are also a field of interest because of their ability to create a smooth surface that is non-toxic to the human body. Nanoparticles are nanomaterials that measure less than 100 nanometers in at least one direction. Commonly researched nanoparticles include copper II oxide, silver, gold, and zinc oxide. The addition of nanoparticles into a known antibiotic enhances the bactericidal effects of the antibiotic (Lara, 2009). Silver shows the most promise in combating infections and antibiotic resistance (Kim, 1994). Silver nanoparticles inhibit the activities of interferon gamma and tumor necrosis factor alpha which are biomarkers of inflammation (Prabhu, 2012).

United States patent US 8647675B2 describes a silver nanoparticle antimicrobial coating for implantable medical devices that can be used for long-term and short-term infection resistance. The coating consists of three distinct layers of silver nanoparticles of different sizes that extend over the surface. A Chinese patent CN 102380130 specifically describes the coating of a modified polyurethane central venous catheter with silver nanoparticles. The process consists of immersion of the catheter in a mixed solvent of ethanol and deionized water before being immersed in a silver nitrate solution by UV irradiation. Nanotechnology is a promising coating mechanism. A thin coating of nanoparticles, approximately 100 nanometers thick, slowly releases silver ions over the course of several days (Roe, 2008). Due to their large surface area, they are more effective. The silver ions bind to the cell wall of the bacteria and, due to their toxicity, cannot be removed (Prabhu, 2012).

Despite showing a reduced risk of infection, nanoparticles in central venous catheters (CVCs) can cause catheter-associated thrombosis in critically ill patients (Stevens, 2009). Nearly 54% of patients in ICUs have a CVC which are associated with an infection rate of 5 per 1000 catheter
days. In critically ill patients, approximately 87% of blood infections are a result of CVC use (Raad, 2011). Primary blood infections from CVCs are caused by various strains of *Streptococcus*, *Staphylococcus*, *Enterococcus*, and *Candida* (Stevens, 2009). *Streptococcus* bacteria cause approximately 36% of blood infections that arise from CVCs (Richards, 1999). In the United States, approximately 3 million CVCs are inserted each year. Approximately 850,000 of CVCs inserted result in an infection, of which 20% are serious and 28% are fatal (Furno, 2004).

Despite the strong antimicrobial properties of silver nanoparticles, these can affect the biocompatibility of blood (Stevens, 2009). However, exposure to metallic nanoparticles over a ten-day period does not pose a toxic risk to the patient. In addition, silver nanoparticles applied to an intravenous catheter continue to release silver ions that inhibit bacterial infections over a ten-day period (Roe, 2008). There is no change in silver concentration over 370 days in urinary catheters and no dangerous levels of silver reach the bloodstream (Samuel, 2004). Silver nanoparticles are a much more viable solution to reduce the risk of infection in urinary catheters compared to broad-spectrum antibiotics.

To ensure biocompatibility, nanoparticles must be biologically synthesized. Chemically synthesized nanoparticles are toxic to humans. If they enter the bloodstream the particles can easily enter the blood-brain barrier. Certain strains of bacteria, plants, and fungi can synthesize silver nanoparticles. Plant extracts are nontoxic, cheaper, and synthesize nanoparticles quickly, and can be used to synthesize nanoparticles for biological use (Prabhu, 2012).

d. Other Current Solutions

Many patented catheter modifications are intended for reducing pathogens entering the body and causing infection. The products often focus on elimination of the rough catheter surface using a coating. Plastic catheters and various coatings exhibit a smoother surface that improves performance and lowers failure rates. Catheter research focuses on preventing infections, fibrin buildup, and thrombosis (blood clots). Several of these techniques can be combined to further reduce the chance of complications from catheter use.

i. Preventing Infections

Improvement of techniques for insertion is centered primarily on proper training and preventative measures such as avoidance strategies, hand hygiene, and daily catheter checks. Proper intervention techniques combat CAUTIs which can be caused by improper insertion techniques. It is important for improvements to occur to increase patient health and decrease costs for hospitals and other healthcare institutions. Direct observation decreases healthcare costs and patient safety, but the most successful strategy to fight CAUTI is to decrease the use of indwelling catheters (Galiczewski, 2017). Due to the necessity of urinary catheters, there is a demand for an improved product.

The use of silicon coated catheters has also been studied as a solution to CAUTIs. Unfortunately, there are no statistically significant data that reveal an advantage to using silicon as a catheter coating (Stenzelius, 2016). Antimicrobial coatings based on antimicrobial peptides are also
suspected to combat CAUTIs. A polyurethane catheter coated with an anti-adhesive hydrophilic polymer coating and antimicrobial peptides prevented bacterial adhesion and inhibited planktonic bacterial growth. These results provide a significant start to understanding the benefits of non-metallic and non-antibiotic coatings for urinary catheters (Yu, 2017).

**ii. Preventing Fibrin Buildup and Thrombosis**

Fibrin is an important protein that is involved in blood clotting. It forms a fibrous mesh that, together with platelets from the bloodstream, impedes blood flow. Fibrin is activated in the final stage of the coagulation cascade, which can be initiated by the presence of foreign substances like catheters. Fibrin adhesion to these surfaces can lead to thrombosis. Fibrin deposits have been shown to increase bacterial colonization of S. aureus (Vanassche, 2013). Antibodies have been developed to prevent the adhesion of S. aureus to fibronectin, but the simplest solution for this problem is catheter replacement (Sun, 1997). Current protocols suggest that intravenous catheters should be changed at least every 3 days, and urinary catheters should be changed at least every 7 days, though daily inspection of the catheter is necessary (Kusek, 2012).

Systematic application of heparin, a blood thinner, prevents fibrin deposition. Intravenous catheters coated with heparin delay fibrin deposition on the outside of catheters (Amplatz, 1971). Heparin medication can be administered during short-term intravenous catheter use but must be administered twice a day and requires constant monitoring to prevent thrombocytopenia (low blood platelet count). Patients with PICC lines must be put on blood thinners such as Prophylaxis with low-dose Coumadin to reduce the risk of thrombosis (Mermel, 2000). Both solutions are effective, but require great care on the part of healthcare professionals. Though catheter replacement reduces the risk of catheter failure and fibrin buildup, it can also increase the risk of infection. Clot formation occurs in both intravenous and urinary catheters, though there are no universally accepted solutions to prevent clot formation with urinary catheter use.

**iii. Ease of Insertion Solutions**

Progress has been made in catheter technology due to new polymers that combine lubricity, softness, and bunch resistance. In addition, the texture and shape of the catheter tip could also play a role in reducing infiltration. Some of the most common strategies to increase ease of insertion include the use of specialized personnel, bevel-down needle insertion in small vessels, the use of local anesthetics, and topical visualization agents. Imaging techniques which assist in vein visualization are also being developed (Helm, 2015).

Ultrasound-guided cannulation (insertion of a thin tube) of deep mid-arm veins by a modified Seldinger technique (US-Seldinger) yields better puncture success rates and lower postoperative complication rates. The technique includes features such as application of pressure with gauze, skin stretching, and immediate delivery of the catheter upon insertion of the catheter sheath. When an ultrasound is used in conjunction with the Seldinger technique, the operator can obtain a clear image of blood vessels which makes it easier to choose an ideal vein for catheter insertion. For this reason, ultrasound guidance provides more precise targeting for needle puncture. The use of the Seldinger technique was also proven to reduce bleeding, pain, complications, and discomfort. Ultrasound equipment used in association with US-Seldinger for a single catheterization operation cost more than the traditional non-US trans-cannula PICC
insertion method, but it has a reduced complication rate. It was concluded that the US-Seldinger technique improves catheter insertion and is suitable for clinical application (Tan, 2016).

The site of insertion has been considered in the fight against pathogens. Insertion of a catheter into the subclavian vein reduces the risk of infection when compared to insertion into the internal jugular vein. Subcutaneous tunneling is an additional catheter insertion technique. According to US patent US4832687, two remote incisions are made and the patented subcutaneous tunneling instrument and method are used to tunnel from one incision to the other. The catheter can then be inserted into the body. However, this technique should not be used if the catheter is intended to draw blood (Mermel, 2000).

Urinary catheters are one of the most uncomfortable medical procedures for patients. As such, many ideas have been implemented to ease the insertion of these devices. The use of lubricant is the primary method used to reduce friction. However, silicone coatings, proper patient compliance, and the use of the smallest possible catheter can also help to make the experience more comfortable for patients (Fink, 2012).

Lubricant helps to minimize the friction between the urethral wall and the catheter. Though its use is vital, often, the lubricant only helps for the first 5 cm of insertion. After that point, the lubricant rubs off. Catheters can be coated in silicone to also minimize the friction force during insertion.

Another factor in patient comfort is their own compliance. The most important factor patients can control is the tension in their muscles. A tense patient will experience more pain. Another nursing technique to ease the insertion of a urinary catheter in a female patient is to position the patient lying flat on their back, with their knees bent. This allows the healthcare provider easy access to the urethra and avoids any unnecessary bending or bunching in the catheter as it is being inserted. In male patients, healthcare providers instruct the patient to lie flat on their back with their legs straight. The key to avoiding unnecessary bending or bunching during catheter insertion in male patients is to hold the penis perpendicular to the body. This position places the urethra in a straighter position, allowing for easier catheter insertion (Fink, 2012).

e. Current Spider Silk Technology

Synthetic spider silk is a tough fibrous material with high bond energy and strength comparable to Kevlar. This biomaterial has biocompatible protein fibers and tunable mechanical properties. The spider in study, *Nephila clavipes*, produces six different types of silk that have unique qualities and purposes for the spider. Harvesting silk from the spiders in large quantities is difficult because they are territorial and cannibalistic. Largescale production of synthetic spider silk has been studied through recombinant protein production.

Although all six of the proteins have been sequenced, there are varying levels of research for each one. The Dragline silk is the strongest, while the Flagelliform has the greatest strain-to-break; these silks are the most understood and studied. Beta-sheet secondary structures are common, highly repetitive motifs found in each of these proteins. These structures create a high crystallinity and play an integral part in the strength of the fibers which, when combined with
other alpha-helices, contribute to the extendibility (Guerette, 2014). These protein structures allow researchers to develop custom proteins that include many of the motifs common in the sequenced Dragline and chimera proteins. Recombinant proteins created for the Dragline silk, namely MaSP1 and MaSp2, are the only proteins that have been expressed in and harvested from goat milk (Hinman, 2000). This is significant as there is limited scalability available in using goat-derived spider silk proteins. Most spider-silk proteins are goat-derived and any major applications will require recombinant proteins scaled in E. coli.

Synthetic spider silk has shown promising results for many medical applications as it does not elicit an immune response, allowing the silk and its products to integrate with tissue. No significant immune response is observed from implanted silk in rats which shows promise for human trials. Synthetic spider silk has also shown improved results in wound closure applications (Hayashi, 1999). Fibroblasts, osteocytes, and mammalian cells can grow on natural spider silk fibers, synthetic spider silk films, and silk hydrogels (Brooks, 2005). Modified silk structures can increase cell proliferation and attachment. Other complementary structures of the synthetic spider silk proteins such as hydrogels, glues, and coatings show promising results in biocompatibility with tunable degradation. Silk films show potential in vaccine storage systems because of decreased bioactivity of the vaccines. Furthermore, silk films can store vaccines at a higher temperature which indicates that a less expensive and stringent storage requirement could be possible (Riekel, 2000).

Synthetic silk production is significantly different from natural fiber production. A spider forms silk protein in individual glands at concentrations of 30-45% weight to volume (w/v) which feed through spinnerets. These proteins are then pulled into a fiber by the back leg of the spider. The protein is secreted and stored in the gland as an aqueous solution and is transformed into a solid fiber as it travels through a fine duct. Synthetic fiber resists solubilizing in water and many other organic solvents. The high concentration found in spider glands has not yet been created in a laboratory setting. When solubilized in hexafluorophosphoric acid, synthetic spider silk reaches concentrations of 12-17% weight to volume.

Aqueous solutions of synthetic spider silk proteins yield lesser concentration. However, the absence of harsh organic solvents increases safety, cost effectiveness, and decreases the environmental impact. Aqueous based synthetic spider silk proteins have been investigated for use as adhesives and coatings. Silk protein based coatings can adhere to numerous substrates (Harris, 2016). Silk materials also possess a smooth surface that does not interfere with blood flow which helps to decrease these inflammatory responses (Hess, 2001). Due to the recombinant nature of the protein and their capability to include other solutes, these coatings can be functionalized for a range of applications. These include prevention of blood clotting and biofouling. This is accomplished by forming drug-eluting coatings that release compounds such as heparin or antibiotics to improve the longevity of intravenous catheters.

There are currently no patents for catheters that incorporates synthetic spider silk technology. However, it is being investigated for other medical applications and a synthetic spider silk coating has been approved for stents. A World Intellectual Property Organization patent WO
2001038373 claims the creation of an improved implantable stent-graft prosthesis to minimize tissue inflammatory responses.

5. Design Process

a. Project Overview

Catheter-related infections are currently combatted using different catheter materials, metal catheter coatings, and antibiotic catheter coatings. The purpose of this project was to create a urinary catheter with antimicrobial properties by introducing a synthetic spider silk protein coating. In this experimental design, a standardized method for uniform synthetic spider silk protein coating has been developed to provide accurate results for all subsequent testing. To produce a product comparable or superior to current catheters in use, specific evaluation criteria were satisfied. AFM and SEM imaging were used to study the roughness of the synthetic spider silk coated catheter and uncoated catheters. The insertion forces of the coated catheters were measured using a vertical MTS to introduce the devices through a layer of silicone material with similar durometer to human skin. Additionally, the antimicrobial properties of the catheter were confirmed using zone of inhibition testing, and the adhesion of the coating to the catheter was observed using a bend and bunch test. A summary of the design decisions made is shown in Figure 7.
Figure 7: Design tree detailing all the design decisions that were developed over the course of the project.
b. Design Steps

i. IV Catheters vs. Urinary Foley Catheters
The first major decision made in this project was deciding to pursue the coating of IV catheters or urinary catheters. At first, IV catheter coatings were pursued. Though synthetic spider silk coatings had been used for IV catheters in the past, the effect of thickness on these coatings had not been explored. The goal of the project would have been to design a method capable of being upscaled to mass production levels. However, faculty advisor Dr. Randolph Lewis clarified that coating thicknesses have little effect on antimicrobial properties. After exploring the catheter coating process previously studied, upsaling to mass manufacturing levels would not require any major design decisions.

Synthetic spider silk coatings for urinary catheters have not been previously investigated. Due to the increasing rate of CAUTI infections and the healthcare industry's prioritization on lowering CAUTI rates, catheter manufacturers are highly interested in antimicrobial coatings. Foley catheters were coated instead of Coudé catheters due to the lack of a hardened tip which simplified the coating process. A summary of the benefits of coating urinary catheters are shown in Table 1.

Table 1: Summary of decisions to coat urinary catheters over intravenous catheters.

<table>
<thead>
<tr>
<th>Catheter Type</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary</td>
<td>Cheaper</td>
<td>Requires more spider silk</td>
</tr>
<tr>
<td></td>
<td>Many design decisions</td>
<td>No established methods</td>
</tr>
<tr>
<td></td>
<td>Easier Imaging</td>
<td></td>
</tr>
<tr>
<td>Intravenous</td>
<td>Method Established</td>
<td>Minimal Designing Involved</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Difficult to image</td>
</tr>
<tr>
<td></td>
<td></td>
<td>More difficult to coat</td>
</tr>
</tbody>
</table>

ii. Synthetic Spider Silk Solution Optimization
Prior to antimicrobial testing with the final catheter samples, initial testing was performed to assess the effect of synthetic spider silk and chlorhexidine, an antiseptic commonly used in medical applications, against three common sources of CAUTIs: *E. coli*, *S. aureus*, and *S. marcescens*. Since chlorhexidine had never been used as part of a coating method it was imperative to know whether it would be beneficial to include as part of the final methods.

Materials
- Liquid Luria-Bertani (LB) media
- Solid LB media with 2% Agar
- Petri dishes
- Sterile Cotton Swabs
- Medical grade silicone
Methods

A 6% synthetic spider silk solution was prepared according to protocol in Appendix a. Medical grade silicone was cut into disks ten millimeters in diameter before sanitization with 70% ethanol. The disks were coated using one of three options: synthetic spider silk, synthetic spider silk + 1.5% chlorhexidine, or 20% chlorhexidine. As a negative control, several disks were left uncoated. Chlorhexidine swabs that are used in hospitals contain 2% chlorhexidine. For the second round of trials, the chlorhexidine was diluted from 20% to 1.5%. The 1.5% chlorhexidine was chosen after examining data from prior trials performed by the faculty mentor, Dr. Lewis.

These trials showed that a 1-2% chlorhexidine solution is ideal. Each disk was dipped in the correct solution for three seconds before it was placed on a paper towel to dry. The disks received the appropriate coating method three times. Once the three coatings were completed, the disks were allowed to dry completely. Agar plates were prepared according to the recipe in Appendix a and streaked with E. coli, S. marcescens, S. aureus. Three plates were streaked for each bacteria species. One plate was the control which contained only the target bacteria. The additional two plates each had four disks containing an uncoated disk and a disk with each of the above coating methods. The plates were incubated inverted at 37°C for 24 hours before zone of inhibition measurements were taken.

Several issues were observed when the plates were checked on the first day. The bacteria were not streaked effectively (the bacteria appeared in grids instead of lawns) and a few of the uncoated silicone disks had moved from the designated grid. An inoculation needle had been used to spread the bacteria on the plates and the needle had gouged the agar in several locations. The results from the first day are shown in Figure 8. It was decided that the initial antimicrobial testing needed to be repeated to resolve the errors that arose.

To ensure proper solvation of synthetic spider silk protein in solution, three mL of the 6% synthetic spider silk solution was prepared for the second round of initial antimicrobial trials instead of two mL. Bacteria were inoculated for 48 hours at 37°C in LB broth instead of agar slants so that cotton swabs could be used to streak the plates in order to form confluent bacterial lawns. Once again, the disks were dipped a total of three times. The same three bacteria (E. coli, S. marcescens, S. aureus) were used for the trials. Inspection of the plates upon the first day of measurements showed the desired bacterial lawn. The zone of inhibition that was measured on the first day was marked with a Sharpie to report a consistent measurement each day. Measurements were recorded every 24 hours for a total of 7 days.

Results

Results from the last day of measurements are shown in Figure 9. All pictures and data are shown in Appendix b. Note that the diameter of the zone measured on the first day are marked with a Sharpie in order to keep measurements consistent. Statistical analysis of antimicrobial data is shown in Tables 2, 3, and 4. Statistical analysis was run using GraphPad Prism using column statistics and one-way analysis of variance (ANOVA) with Tukey HSD post-hoc test. Significance when compared to the uncoated control has a p-value less than 0.05.
Figure 8: Picture of plates after the first day of zone of inhibition measurements. (1) From top to bottom: S. marcescens control plate, S. marcescens replicate plate one, S. marcescens replicate plate two. (2) From top to bottom: S. aureus control plate, S. aureus replicate plate one, S. aureus replicate plate two. (3) From top to bottom: E. coli control plate, E. coli replicate plate one, E. coli replicate plate two. On all replicate plates starting from the top left and moving clockwise: uncoated disk, synthetic spider silk coated disk, synthetic spider silk + 1.5% chlorhexidine disk, and 1.5% chlorhexidine disk. Note that several disks moved from the designated quadrant.
Figure 9: Picture of plates after the seventh day of zone of inhibition measurements. (1) From top to bottom: E. coli control plate, E. coli replicate plate one, E. coli replicate plate two. (2) From top to bottom: S. aureus control plate, S. aureus replicate plate one, S. aureus replicate plate two. (3) From top to bottom: S. marcescens control plate, S. marcescens replicate plate one, S. marcescens replicate plate two. On all replicate plates starting from the top left and moving clockwise: uncoated disk, synthetic spider silk coated disk, synthetic spider silk + 1.5% chlorhexidine disk, and 1.5% chlorhexidine disk.
Table 2: Statistical analysis for the disks using the seventh day zone of inhibition measurements for E. coli.

<table>
<thead>
<tr>
<th></th>
<th>Average (mm)</th>
<th>Standard Error of the Mean</th>
<th>Standard Deviation</th>
<th>Lower 95% Confidence Interval of Mean</th>
<th>Upper 95% Confidence Interval of Mean</th>
<th>Significant Compared to Uncoated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoated</td>
<td>10.00</td>
<td>0.00</td>
<td>0.00</td>
<td>10.00</td>
<td>10.00</td>
<td>No</td>
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<tr>
<td>Synthetic Spider Silk</td>
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<td>0.75</td>
<td>1.06</td>
<td>3.22</td>
<td>22.28</td>
<td>Yes</td>
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<tr>
<td>Synthetic Spider Silk + Chlorhexidine</td>
<td>18.00</td>
<td>0.00</td>
<td>0.00</td>
<td>18.00</td>
<td>18.00</td>
<td>Yes</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>17.50</td>
<td>0.50</td>
<td>0.71</td>
<td>11.15</td>
<td>23.85</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Table 3: Statistical analysis for the disks using the seventh day zone of inhibition measurements for S. aureus.

<table>
<thead>
<tr>
<th></th>
<th>Average (mm)</th>
<th>Standard Error of the Mean</th>
<th>Standard Deviation</th>
<th>Lower 95% Confidence Interval of Mean</th>
<th>Upper 95% Confidence Interval of Mean</th>
<th>Significant Compared to Uncoated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoated</td>
<td>10.00</td>
<td>0.00</td>
<td>0.00</td>
<td>10.00</td>
<td>10.00</td>
<td>No</td>
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<tr>
<td>Synthetic Spider Silk</td>
<td>14.50</td>
<td>0.50</td>
<td>0.71</td>
<td>8.15</td>
<td>20.85</td>
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<tr>
<td>Synthetic Spider Silk + Chlorhexidine</td>
<td>28.50</td>
<td>5.50</td>
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<td>-41.38</td>
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<tr>
<td>Chlorhexidine</td>
<td>32.00</td>
<td>6.00</td>
<td>8.49</td>
<td>-44.24</td>
<td>108.20</td>
<td>Yes</td>
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</table>

Table 4: Statistical analysis for the disks using the seventh day zone of inhibition measurements for S. marcescens.

<table>
<thead>
<tr>
<th></th>
<th>Average (mm)</th>
<th>Standard Error of the Mean</th>
<th>Standard Deviation</th>
<th>Lower 95% Confidence Interval of Mean</th>
<th>Upper 95% Confidence Interval of Mean</th>
<th>Significant Compared to Uncoated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoated</td>
<td>10.00</td>
<td>0.00</td>
<td>0.00</td>
<td>10.00</td>
<td>10.00</td>
<td>No</td>
</tr>
<tr>
<td>Synthetic Spider Silk</td>
<td>12.00</td>
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<td>1.41</td>
<td>-0.71</td>
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<tr>
<td>Synthetic Spider Silk + Chlorhexidine</td>
<td>20.00</td>
<td>1.00</td>
<td>1.41</td>
<td>7.29</td>
<td>32.71</td>
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<tr>
<td>Chlorhexidine</td>
<td>21.50</td>
<td>4.50</td>
<td>6.36</td>
<td>-35.68</td>
<td>78.68</td>
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Conclusions

It was decided that sterile cotton swabs needed to be used in all further experiments to create the bacterial lawn due to the grid-like appearance shown in Figure 8. Figure 9 shows the confluent lawns that were created when using the cotton swab. The disks that were coated with chlorhexidine showed successful zones of inhibition and were included in the final catheter
coatings. Chlorhexidine was shown to enhance the antimicrobial properties of the coating solution and was later used in trials with Foley catheters.

**iii. Catheter Coating**

To test the mechanical and antimicrobial properties of the coated catheters, coating methods had to be optimized. Previous results in literature suggested methods such as dip and spray coating (Lewis, 2016). Based on suggestions from the faculty mentor and industry representatives, an aerosolize coating was developed along with coatings combining methods. Based on the qualitative results from synthetic spider silk solution optimization, coatings were made using synthetic spider silk and synthetic spider silk with 1.5% chlorhexidine solution.

1. Dip Coating

*Materials*
- Polycarbonate (thickness of 0.6 cm)
- Loctite Plastic Epoxy
- Loctite Super Glue (Cyanoacrylate)
- 6% w/v Synthetic Spider Silk Solution
- 20% Chlorhexidine Solution
- Latex Urinary Catheters
- Stren Super Knot Monofilament Fishing Line
- Dritz Yarn Darners
- 10% Bleach Solution
- 20% isopropyl alcohol (IPA)

*Methods*

A volume of six mL of synthetic spider silk solution was prepared according to the protocol in Appendix a. A trough of polycarbonate was made with the dimensions 19.8 cm x 1.5 cm x 2 cm using epoxy and cyanoacrylate (Figure 10). The trough held 59.4 mL of solution. A minimum of 20 mL of solution were needed to submerge the catheter which was impractical and wasteful. To decrease the necessary volume of spider silk, an insert piece of polycarbonate was added to the trough (Figure 11). The new dimensions of the trough were 6.5 cm x 1.5 cm x 2 cm. To submerge the catheter in this new setup, approximately 6 mL of solution were sufficient. It was discovered that using more than six mL of solution at a time caused the solution to gel before the coating process was finished.

Uncoated catheters were pierced through one of the forked ends with a needle, then a length of fishing line was pulled through the hole to tie a loop, while another length of fishing line was used to create a loop at the tip of the catheter (Figure 12). The loops were used to pull the catheter through the trough to avoid unnecessary contact. With the smaller trough, touching the catheter while coating was unavoidable. Contact was kept to just below the forked end and at the very tip where the coating would drip down while drying.

Prior to coating, the catheters and the trough were sterilized in a 10% bleach solution for 10 minutes then rinsed with 20% IPA. The sterilization step was required to prevent contamination during the antimicrobial trials. All 6 mL of solvated synthetic spider silk or synthetic spider silk
solution with chlorhexidine were pipetted into the trough. The catheter was pulled through solution, rotated, and then pulled back through to ensure full coverage. Once both passes were complete, the catheter was hung from a ring stand to dry for approximately five minutes (Figure 13). A total of three coatings were deemed necessary based on literature (Lewis, 2016).

A blue food coloring was added to the solution during some initial trials to easily visualize the coating. The coloring made it easier to see the bubbles trapped on the surface of the catheter from bubbles that formed while pipetting the solution into the trough. This was mitigated by pipetting very slowly into the trough.

Based on industry needs, the team attempted to create an inside coating using the dip method. The catheter was pulled through the trough, the opening on the forked end allowed the coating solution into the catheter. Excess solution was then pipetted into the catheter while hanging from the drying apparatus, moving the catheter around the inside to assist in a consistent coating. The coating then dripped out of the opening in the tip of the catheter.

Figure 10: The polycarbonate trough.

Figure 11: Insert addition to reduce volume of trough.
Figure 12: Looped fishing line to pull the catheter and hang to dry.

Figure 13: The apparatus to hang dry the catheters.

Conclusion
The dip coating method left a visible coating on the catheters, but the food coloring made the bubbles on the surface stand out. The catheters needed to dry after coating for several hours, but the catheters remained sticky after drying. The dip-coated catheters made a crinkling sound when bent.

The inside of the catheter near the forked opening appeared to be evenly coated; however, later SEM imaging revealed that the inside was not consistently or evenly coated.
2. Aerosolize Coating

Materials

- 6% w/v Spider Silk Solution
- 20% Chlorhexidine solution
- Isopropyl Alcohol (IPA)
- Ultrasonic Lumiscope Nebulizer (Model 6700)
- Salter Labs Nebulizer (Model: 8900-7)
- Glass vacuum adapter
- Vinyl hose, 1" O.D.
- Urinary catheters

Methods

A two mL solution of 6% (w/v) synthetic spider silk solution was prepared in a glass vial via protocol in Appendix a. The solution was pipetted into a portable Lumiscope ultrasonic nebulizer to coat a catheter sterilized in a 10% bleach solution and rinsed with IPA. The first coating attempt was aimed at coating the inside surface of the catheter. A glass vacuum adapter was purchased in order to connect to the vacuum outlet in the fume hood. The catheter was attached to one end of the adapter and held by hand in the other. The vacuum and nebulizer were turned on and the nebulizer nozzle was focused into the tip of the catheter. The aerosolized synthetic spider silk solution was difficult to see which made it impossible to determine if the inside of the catheter was coated effectively. It appeared that the synthetic spider silk was not exiting the nebulizer in a continuous stream and upon inspection it was determined that the solution had solidified. The Lumiscope model that was being used relied on an outlet and could handle a maximum of fifteen watts of power. Unfortunately, the nebulizer was not strong enough to aerosolize the solution.

The team decided to reduce the percentage of synthetic spider silk in solution to 3% (w/v) to ensure release by the nebulizer and to find a new nebulizer that relied on an air source instead of a battery. A new nebulizer was purchased from Alpine Medical made by Salter Labs (Model: 8900-7). An extra vinyl hose (1" O.D.) was purchased with the idea of creating a "T" shape with the existing hose. The catheter could then be placed through the two hoses which would allow the synthetic spider silk solution to aerosol down the two hoses and across the entire catheter. After discussing this idea, it was discarded for three different reasons. First, the vinyl hose that was purchased was too small to fit around the "T" attachment of the nebulizer. Second, the synthetic spider silk is only released out of one side of the "T". This means that only one side of the catheter would be coated with synthetic spider silk. Lastly, the catheter would not be coated evenly. The middle of the catheter would have a thicker coating than the two ends because the synthetic spider silk meets the middle first before moving outward more slowly.

The next coating trials were performed using the new nebulizer with a 3% (w/v) and 6% (w/v) synthetic spider silk solution. The air connection was only available on a lab bench so the coating was performed outside of the fume hood. The catheter was hung from a ring stand with fishing line and the nebulizer head was passed down the catheter and back up. This up and down coating motion was repeated rotating around the entire catheter. Both the 3% and 6% solutions
were aerosolized and sprayed onto the catheter with two coatings with a ten-minute break in between for drying.

Although the new nebulizer successfully released the spider silk as an aerosol, it was difficult to determine whether the synthetic spider silk was adhering to the catheter. During the next coating trial, red food coloring dye was added to the synthetic spider silk solution after centrifugation and before it was pipetted into the medicine cup to help visualize attachment of the synthetic spider silk to the catheter. The nozzle head was also turned 90 degrees so that it was parallel to the hanging catheter instead of perpendicular as in the previous trial. This directed the aerosolized synthetic spider silk up and down the catheter.

Previously, the solution was moving around the catheter which reduced the amount of surface area that was coated. A three mL solution was used for a total of three coatings. There was an obvious red discoloration to the nebulizer hose and attachment but not to the catheter. An additional three mL of synthetic spider silk solution was used to coat the catheter for three more coatings. Upon inspection, there appeared to be a change in color to the catheter as well as a matte finish that contrasted with the initial shiny appearance of the surface.

Antimicrobial testing with samples from the coated catheters verified coating results from the nebulizer trials and results are discussed in the antimicrobial testing section. The 3% synthetic spider silk coating had no effect on antimicrobial properties. Therefore, it was decided not to continue with this coating method. The 6% synthetic spider silk coating showed inhibition to bacteria growth but the coating was not as effective as other coating methods such as dip coating. Using the nebulizer as a coating method is still a viable option but serves better as a secondary coating method. Since antimicrobial results showed that a combination of coating methods seems to be the most effective at inhibiting bacterial growth, a new coating option was created: dip coat with a secondary aerosolize coat.

It was also decided to attempt an inside catheter coating with the nebulizer. To accomplish this, a nozzle apparatus was created by the team and is shown in Figure 14. Cake decorating tips were attached to one end of the hose using tape so that the tip could be inserted into the open end of the catheter (Figure 15). The other end of the catheter was attached to the glass vacuum adapter using piping bags and tape (Figure 16). The nebulizer was connected to the air source on the bench top and the vacuum adapter was attached to the vacuum source in an attempt to create a continuous flow of aerosolized synthetic spider silk solution through the catheter. The vacuum pulled the air through the catheter and cooled it down which caused the synthetic spider silk solution to aerosolize. This was visible when the nozzle was removed from the catheter and led us to believe that the inside coating had coagulated. In order to determine if this was the case, SEM imaging was performed for this method. SEM images of the inside coating are shown in a later section.
Figure 14: Image of apparatus set up for the inside aerosolize coating method.

Figure 15: Image of the nozzle that was designed for the inside aerosolize coating method.
Figure 16: Image showing the vacuum adapter set up for the inside aerosolize coating method.

Figure 17: Image that shows coagulation of the spider silk after an attempt to coat the inside of the catheter.
Conclusions
The aerosolize coating method is very straight forward and easy to set-up. However, the synthetic spider silk aerosol that is released by the nebulizer creates a thin coating. Because of this, it is recommended that the aerosolize coating method be used as a secondary coating method if a thicker coating is desired.

The catheter felt cool to the touch when the above-mentioned protocol was used in an attempt to coat the inside. As shown in Figure 17, the synthetic spider silk solution precipitated from solution and formed beads. This led to the assumption that if the inside was coated it was most likely an uneven surface of coagulated spider silk solution.

3. Spray Coating

Materials
- 6% w/v Spider Silk Solution
- 20% Chlorhexidine solution
- Airbrush Master Series II
- DACA Winder
- Steel Rod 70 cm in length
- Urinary Catheters

Methods
Before coating, the apparatus used to generate uniform rotational velocity was prepped. The DACA winder featured a steel bar that was 25 mm in diameter and 220 mm long. Approximately 40 mm of the bar at the suspended end was threaded. The mounted end is connected to the motor that rotates the bar at tunable velocities. A 3D printed mount was designed in SolidWorks and constructed of polylactic acid. It included a hollow cylindrical end with a 45 mm outside diameter, a 19 mm inside diameter, and is 40 mm long. The center of the mount was originally designed as a transitional piece with a stepped cut to accommodate different sized catheters (Figure 18). This design was modified when the decision to no longer test IV catheters was made. A tapped hole in the fixture threaded the hollow end of the mount with grooves to match those of the steel bar. Once the grooves were tapped into the mount it was placed onto the steel rod by screwing the threaded pieces together. The catheter was cut and attached to the small diameter until it fit tightly and was centered on the winder (Figure 18).

The DACA winder was a key element in the spray coating method because its constant rotation promotes continuous coating across the catheter surface. An issue arose in attempting to attach Foley catheters to the rotational piece. It was impossible to maintain a permanent position for the Foley catheter so a fixture was designed to fit on the DACA winder rotating arm with a snug fit for catheter hubs to be inserted to. To create such a fixture, dimensions of the winder were measured using calipers to determine the diameter, length, peak length and pitch of the threads. The drawing seen in image Figure 19 was the initial design for a mounting solution. The final design was then sent to the USU 3D printing office using ABS plastic in a low density setting to decrease the amount of overall material used in the printing process.

Exact dimensions were difficult to obtain so plumber’s tape was wrapped around the threaded portion of the DACA winder rotating arm several times to thicken the diameter and fill in the
gaps (Figures 20 and 21). This served to further secure the mount and ultimately proved as a successful solution to mounting IV catheters.

The fixture to be added to the mounting assembly was designed in a way that it could be inserted into the original DACA winder mount. This converted the initial part from mounting IV catheters to being able to mount Foley catheters (Figure 22). The final design was then sent to the USU 3D printing office using ABS plastic in a low density setting to decrease the amount of overall material used in the printing process (Figure 23).

After the mounts were completed and assembled the first coatings began. Once the DACA Winder was started, the “Winder Only” option on the display was selected. The prompt to enter the spool diameter (outside diameter of catheter) in millimeters and 30mm was entered. The winder speed was set at 6 meters per second (m/s). The last prompt was to choose the step size of each velocity increase which was set at 1 m/s. These parameters were not chosen to be accurate to the diameter of the catheter. They were instead chosen as a benchmark for all the catheters to be coated at the same speed. It was imperative that the winder spin quickly enough to get a thin even coating, and that the rotational speed (winder settings) was uniform throughout coatings. Before winding, the catheter was to be marked with a pen to distinguish the start and stop positions for the air brush.

Using the Airbrush Master Series II 6% synthetic spider silk solution was loaded into the liquid chamber of the airbrush gun. The airbrush features a small air compressor and mechanical settings designed to tune the quantity of liquid allowed to disperse into the otherwise constant airflow. Of course this airflow was only constant after it had been allowed to dispense excess pressure. Because of this, the catheter was only sprayed after the gun had sprayed for several seconds. The catheter was then moved at a constant lateral velocity along the entire length of the catheter. The catheter was promptly removed using sterile forceps and secured to dry vertically.

Upon coating the first several catheters, it was discovered that the lateral speed was not consistent. The DACA Winder features a speed variable guide that travels 14 cm back and forth parallel to the catheter. However, the guide is offset about 30 cm from the position of catheter being spun. A steel rod used in the drying apparatus was placed on the operator’s shoulder and the other end on a clamp attached to the sliding guide. The rod would move at a constant velocity with the guide and slide freely across the shoulder the airbrush would be placed and held at a constant position along the steel rod. This limited lateral movement of the airbrush to the velocity preset by the sliding guide. By keeping the preset velocity the same, uniform lateral movement was permitted across all samples. The limited 14 cm tract of the guide also meant that the position of the airbrush on the steel rod would need to change to cover the entire length of the catheter. This required careful observation of where the spray coating ended to align that position to the spray coating coming from the other direction. Due to human error, double coating for this small segment of the catheter was inevitable.

Further issues arose with the mechanical settings on the airbrush that moved slightly with the vibration of the air compressor and would not return to the exact same setting after each use. This meant the settings were adjusted after each spin and there was no way to determine if there were an equal flow rate between trials except to allow the airbrush to spray a glove and the rate
at which the glove was wetted was observed and adjusted so that it would be similar each time. The instruments available did not allow measurement of these flow rates to ensure uniformity over each coating.

Figure 18: Depiction of initial iteration for 3D printed mount for use in catheter spray coating procedures. This design had originally considered coating IV catheters and was discarded due to the design change from IV catheters to Foley catheters.

Figure 19 First edition of 3D printed catheter mount for DACA winder. This second design iteration was discarded because it did not fit the dimensions of the DACA winder and threads were deemed a necessary design addition.
Figure 20: Final edition of 3D printed catheter mount for DACA winder. Threading was added in the final design to allow for a more secure fit. Accurate thread dimensions were difficult to obtain from the DACA winder and so plumbing tape was wrapped around the threaded portion to fill in spaces between the printed part and instrument.

Figure 21: Final iteration of 3D printed spray coating mount secured on DACA Winder. An additional fixture was later deemed necessary to allow a dowel to be inserted upon which the Foley catheter would be secured.
Figure 22: Complete assembly of 3D spray coating fixture, additional dowel fixture, and dowel held securely on DACA winder.

Figure 23: Details for 3D dowel fixture which was designed to allow a metal rod to be attached to the winder. The catheters were slid over the rod to ensure smooth and consistent rotation.
Conclusions
The spray coating method required several moving parts and dried almost immediately upon contact due to the use of compressed air as a mobilizing spray. The coating was difficult to observe with the naked eye, and SEM imaging was performed to ensure the spray technique provided a uniform coating. Images confirmed that the coatings were present and smooth. Inner coatings were not feasible and were not attempted with this method.

4. Spray and Dip Coating
Materials
- 6% w/v Synthetic Spider Silk Solution
- 20% Chlorhexidine Solution
- Latex Urinary Catheters, Spray coated

Methods
After a catheter was coated using the spray coating method, the catheter was allowed to dry for 24 hours. The catheter was then dip coated using the dip coat protocol described previously. The catheters were sterilized prior to spray coating however, no sterilization took place before the dip coating. If the first coating contained chlorhexidine, the second layer also contained chlorhexidine.

Conclusion
The spray coated catheter became sticky after dip coating. There was less of a crinkling when the spray and dip coated catheter was bent, but there was still visible cracking in the coating.

5. Dip and Aerosolize Coating
Materials
- 6% w/v Synthetic Spider Silk Solution
- 20% Chlorhexidine Solution
- Latex Urinary Catheters, Dip coated

Methods
A catheter was dip coated and allowed to dry for 24 hours. The catheter was then coated using the aerosolize method described above. If the first coating method contained chlorhexidine, the second layer also had chlorhexidine.

Conclusion
After dip coating the catheter had a shine to the surface; after the additional aerosolize coating, the catheter was more matte in appearance. There was less of a crinkling sound when the catheter was bent as compared to the dip method. However, there were more cracks that showed in the dip and aerosolize coating compared to just an aerosolized coating.
iv. Antimicrobial Testing with Coated Catheters

The antimicrobial effect of the catheter coatings needed to be measured in order to meet the specified design criteria. Zone of inhibition testing served as a quantitative test that allowed for a final catheter design decision. The bacteria used in initial testing (E. coli, S. aureus, and S. marcescens) were included in the final trials with catheters coated by the optimized coating protocols.

Materials
- Liquid Luria-Bertani (LB) media
- Solid LB media with 2% Agar
- Petri dishes
- Sterile cotton swabs
- Catheters coated with optimized coating protocols

Methods
Antimicrobial testing was performed once catheters were coated with all the final coating methods. A practice plate was inoculated with different sample shapes to determine which shape would stay attached to the agar during incubation. Scissors were used to cut an oblong shape and a scalpel was used to cut a sample of the cross-section (ring) and a rectangle from a dip coated catheter. A plate was inoculated with S. aureus bacteria and put in the incubator for 24 hours. As seen in Figure 24, the oblong sample fell off but the ring and rectangle stayed attached to the agar. The ring showed more uniform inhibition of bacterial growth when compared with the rectangle sample. Due to this, it was decided that final antimicrobial testing would be accomplished using a cross-sectional area sample from each catheter. Problems occurred when the catheters were cut for testing. The silicone catheters were difficult to cut which resulted in samples that were not completely smooth and did not lay flat on the agar. To combat this problem, the samples were gently pressed into the agar once they were deposited.

The zone of inhibition test was performed using E. coli, S. aureus, and S. marcescens. A control plate was inoculated for each bacteria species. Then, each catheter sample was placed on an inoculated plate. Each coating method was plated twice. The following is a list of the catheter samples that were used for testing: 6% synthetic spider silk dip coat, 6% synthetic spider silk spray and dip coat, 6% synthetic spider silk spray coat, 6% synthetic spider silk with 1.5% chlorhexidine solution, 6% synthetic spider silk aerosolized coat, 3% synthetic spider silk aerosolized coat, and an uncoated catheter. Zones of inhibition were measured every day for a total of seven days. Despite pressing the samples into the agar a few samples fell off during the week of testing which required additional antimicrobial testing.

During the first antimicrobial trial, a dip and aerosolized coating method was created. Catheters were coated with both a 6% synthetic spider silk solution and 6% synthetic spider silk with 1.5% chlorhexidine solution. The samples from the new coating method were added to the second trial of antimicrobial testing. The spray coating protocol was further optimized which required new samples to be incorporated into antimicrobial testing. Ultimately, the second round of testing
consisted of any catheter samples that fell off during the previous round, samples from the new coating methods, and samples from a catheter coated with the optimized spray protocol.

To obtain the necessary number of measurements to ensure proper statistical analysis, a third and final trial of antimicrobial testing was set up. Samples from all five coating methods with both 6% spider silk and 6% spider silk and 1.5% chlorhexidine were included.

Results
Images for the seventh day of testing for all trials are shown below in Figures 25 through 30. A scale bar has been added to all pictures for reference. All pictures are available in Appendix b. The average zone of inhibition in millimeters is shown for each coating method at day seven for each bacterium in Tables 5, 6, and 7 below along with the 95% confidence interval values, standard deviation, and the standard error of the mean. All measurements for the three bacteria used can be seen in Appendix b. The values that are shown in the table below were used to create Figures 31, 32, and 33 respectively. Statistical analysis was run using GraphPad Prism using column statistics and one-way analysis of variance (ANOVA) with Tukey HSD post-hoc test. Significance when compared to the uncoated control has a p-value less than 0.05.

Figure 24: Picture showing the different sample shapes that were tried to determine the best one for antimicrobial testing. From top moving clockwise: cross-section (ring), rectangle, and oblong.
Figure 25: Pictures of zones of inhibition from trial 1 for samples of coating methods against E. coli. Descriptions start in the top left quadrant and continue clockwise for each plate. (1,2) 6% spider silk dip, 6% spider silk + 1.5% chlorhexidine dip, 6% spider silk + 1.5% chlorhexidine spray and dip, and 6% spider silk spray and dip. (3,4) 3% spider silk aerosolized, 3% spider silk + 1.5% chlorhexidine aerosolized, 6% spider silk + 1.5% chlorhexidine spray, and 6% spider silk spray. (5,6) 6% spider silk aerosolized, 6% spider silk + 1.5% chlorhexidine aerosolized, and uncoated.

Figure 26: Picture of zones of inhibition from trial 1 for samples of coating methods against S. aureus. Descriptions start from the top left quadrant and continue clockwise for each plate. (1,2) 6% spider silk dip, 6% spider silk + 1.5% chlorhexidine dip, 6% spider silk + 1.5% chlorhexidine spray and dip, and 6% spider silk spray and dip. (3,4) 3% spider silk aerosolized, 3% spider silk + 1.5% chlorhexidine aerosolized, 6% spider silk + 1.5% chlorhexidine spray,
and 6% spider silk spray. (5,6) 6% spider silk aerosolized, 6% spider silk + 1.5% chlorhexidine aerosolized, and uncoated.

Figure 27: Picture of zones of inhibition from trial 1 for samples of coating methods against S. marcescens. Descriptions start from the top left quadrant and continue clockwise for each plate. (1,2) 6% spider silk dip, 6% spider silk + 1.5% chlorhexidine dip, 6% spider silk + 1.5% chlorhexidine spray and dip, and 6% spider silk spray and dip. (3,4) 3% spider silk aerosolized, 3% spider silk + 1.5% chlorhexidine aerosolized, 6% spider silk + 1.5% chlorhexidine spray, and 6% spider silk spray. (5,6) 6% spider silk aerosolized, 6% spider silk + 1.5% chlorhexidine aerosolized, and uncoated.

Figure 28: Picture of zones of inhibition from trial 2 for samples of coating methods against E. coli. Descriptions start in the top left quadrant and continue clockwise for each plate. (1,2) 6% spider silk spray, 6% spider silk + 1.5% chlorhexidine spray, 6% spider silk + 1.5% chlorhexidine dip and aerosolized, 6% spider silk dip and aerosolized. (3) 6% spider silk + 1.5% chlorhexidine aerosolized (sample 1), 6% spider silk + 1.5% chlorhexidine aerosolized (sample 2), and 6% spider silk + 1.5% chlorhexidine spray and dip. (4) 6% spider silk aerosolized and 6% spider silk + 1.5% chlorhexidine dip and aerosolized.
Figure 29: Picture of zones of inhibition from trial 2 for samples of coating methods against *S. aureus*. Descriptions start in the top left and continue clockwise for each plate. (1) 6% spider silk spray, 6% spider silk + 1.5% chlorhexidine spray, 6% spider silk + 1.5% chlorhexidine dip and aerosolized, 6% spider silk dip and aerosolized. (2) 6% spider silk spray, 6% spider silk + 1.5% chlorhexidine spray, 6% spider silk + 1.5% chlorhexidine dip and aerosolized, 6% spider silk dip and aerosolized.

Figure 30: Picture of zones of inhibition from trial 2 for samples of coating methods against *S. marcescens*. Descriptions start in top left and continue clockwise for each plate. (1) 6% spider silk spray, 6% spider silk + 1.5% chlorhexidine spray, 6% spider silk + 1.5% chlorhexidine dip and aerosolized, 6% spider silk dip and aerosolized. (2) 6% spider silk spray, 6% spider silk + 1.5% chlorhexidine spray, 6% spider silk + 1.5% chlorhexidine dip and aerosolized, and 6% spider silk dip and aerosolized. (3) 6% spider silk aerosolized and 6% spider silk + 1.5% chlorhexidine spray and dip.
Table 5: Statistical analysis for the seventh day zone of inhibition measurements for *E. coli*.

<table>
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<tr>
<th>Treatment</th>
<th>Average (mm)</th>
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<th>Standard Deviation</th>
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<th>Upper 95% Confidence Interval of Mean</th>
<th>Significant Compared to Uncoated</th>
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Table 6: Statistical analysis for the seventh day zone of inhibition measurements for *S. aureus*.

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Table 7: Statistical analysis for the seventh day zone of inhibition measurements for S. marcescens.

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<td>Aerosolize</td>
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<td>0.00</td>
<td>0.00</td>
<td>8.00</td>
<td>8.00</td>
<td>No</td>
</tr>
<tr>
<td>Aerosolize + Chlorhexidine</td>
<td>8.00</td>
<td>0.00</td>
<td>0.00</td>
<td>8.00</td>
<td>8.00</td>
<td>No</td>
</tr>
<tr>
<td>Dip and Aerosolize</td>
<td>8.00</td>
<td>0.00</td>
<td>0.00</td>
<td>8.00</td>
<td>8.00</td>
<td>No</td>
</tr>
<tr>
<td>Dip and Aerosolize + Chlorhexidine</td>
<td>11.27</td>
<td>0.65</td>
<td>2.15</td>
<td>9.83</td>
<td>12.72</td>
<td>Yes</td>
</tr>
<tr>
<td>Spray and Dip</td>
<td>8.00</td>
<td>0.00</td>
<td>0.00</td>
<td>8.00</td>
<td>8.00</td>
<td>No</td>
</tr>
<tr>
<td>Spray and Dip + Chlorhexidine</td>
<td>11.67</td>
<td>0.49</td>
<td>1.21</td>
<td>10.40</td>
<td>12.94</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Figure 31: Data shown are the average zones of inhibition ± standard deviation for each coating method against E. coli. Each number corresponds to one of the spider silk coating type (1 = spray, 2 = dip, 3 = aerosolize, 4 = dip and aerosolize, and 5 = spray and dip) and the addition of “C” represents coatings containing chlorhexidine. Different letters indicate groups are statistically different (p < 0.05) from the control according to ANOVA with Tukey HSD post-hoc tests (GraphPad Prism). To keep the figures easy to read, significance when compared between coating methods was not recorded. Significance between coating methods is detailed in Appendix f.
Figure 32: Data shown are the average zones of inhibition ± standard deviation for each coating method against *S. aureus*. Each number corresponds to one of the spider silk coating type (1 = spray, 2 = dip, 3 = aerosolize, 4 = dip and aerosolize, and 5 = spray and dip) and the addition of “C” represents coatings containing chlorhexidine. Different letters indicate groups are statistically different (p < 0.05) from the control according to ANOVA with Tukey HSD post-hoc tests (GraphPad Prism). To keep the figures easy to read significance when compared between coating methods was not recorded. Significance between coating methods is detailed in Appendix f.
**Conclusions**

The results collected from the antimicrobial trials showed that spider silk alone does not provide a sufficient antimicrobial coating. The samples coated with synthetic spider silk and chlorhexidine showed much greater zones of inhibition than the samples that did not. Statistical analysis showed that the dip with chlorhexidine, dip and aerosolize with chlorhexidine, and spray and dip with chlorhexidine coatings exhibited significantly larger zones of inhibition than the other coating methods when compared to the uncoated catheter.

**v. Friction Testing**

The addition of a coating to the exterior of a urinary catheter will either increase or decrease the amount of force required to insert the device into the urethra of a patient. For this reason, a friction test was deemed necessary to evaluate the change in mechanical properties of coated catheters versus uncoated ones. The goal of this test was to prove that adding a layer of synthetic...
spider silk coating will either decrease the resistant force/friction of silicon with skin, or maintain the same amount of force and demonstrate that the coating does not adversely affect the mechanical properties of a catheter. Several methods and approaches were attempted before finding a protocol that accurately measured the differences in friction between catheters accurately.

**Materials**
- 8-32 six-inch-long dowel rod
- 8-32 nut
- 10-32 one inch bolt
- 10-32 washer
- 10-32 nut
- MTS tensile tester (lateral and vertical)
- 10 N load cell
- Pneumatic grips
- TestWorks 4 Software
- High Strength High Temperature Silicon Rubber Sheet (1/16”)
- Scissors
- Marking pen
- 2 large clamps
- 5 stackable height test tube pieces
- Copper pipe with set screws
- Hex wrenches
- Needle nose pliers
- Punch press set
- Hammer
- Fishing line
- Size 2 sewing needle
- 4”x 1” scrap metal

**Method 1**
Several pieces of the silicon sheet were cut into one inch square pieces using scissors and punched through the center using a punch press set and hammer. This created an 11-mm diameter hole. The squares were then stacked to a height of approximately four inches and laced together using fishing line and a size two sewing needle. This fixture was made to simulate a human urethra. A 10-32 bolt and corresponding washer were attached at the end of the four-inch fixture and sewn on with 4 additional squares. A 10-32 nut kept the bolt secure within the silicon urethra. This fixture was then mounted to the lateral MTS and a catheter was placed in the pneumatic grips on the opposite side. As the load cell detracted, it measured the amount of force necessary to remove the catheter from the urethra.

**Changes to Method 1**
The urethra was too flexible under its own weight upon attachment to the lateral MTS and could not maintain a horizontal position to allow for accurate measurements. A piece of sheet metal scrap was bent to the outer shape of the urethra and a hole was extruded from the side to attach at
the bolted end of the urethra and provide further support. The supported fixture could maintain its horizontal alignment; however, the force of the catheter being pulled outward still bent the silicon urethra downward slightly and created unusable measurements (Figure 34). Ultimately, the lateral MTS was deemed a non-viable option because of the variability between each measurement.

![Figure 34: Profile view of silicon urethra attached to lateral MTS. Slight bending of the fixture can be seen from the desired 180° position.](image)

**Method 2**

This second method utilized the silicon urethra assembled in method one, however the vertical MTS was used instead (Figure 35). This reduced variability between catheter trials and created more stability in the overall assembly. An 8-32 six-inch dowel was inserted into the catheter to ensure that for each test run, the catheter moved over the same path as the previous trial and output. The urethra was placed on the base of the MTS and the catheter was gradually pulled out of the fixture. To do this, the dowel inserted into the catheter was attached to the 10 N load cell using a copper pipe with set screws to convert the 8-32 diameter bolt to the appropriate size for the MTS.

**Changes to Method 2**

The data from this set up was still too variable due to ineffective clamps attached to the load cell. Several shims were placed to create a tighter fit and the positions of the silicon fixture and catheter assembly were switched. The 8-32 dowel was bolted to the base and the urethra was attached to the clamps of the load cell and moved up and down to simulate an insertion procedure. However, the program still did not output consistent results.
Method 3

It was made apparent by methods one and two that perhaps the interior of the silicon urethra was not completely uniform and could be the cause of so much variation between test samples. The fixture was therefore not included in method 3. This third method utilized the remaining sheet of silicon to simulate the force a catheter needs to overcome the friction coefficient between the surface of the catheter and the urethra of a patient. Continuing to use the vertical MTS, five stackable test tube holders were placed on the base of the MTS and held in place by two large table clamps (Figure 36). A 9-mm hole was punched through the silicon sheet using a hammer and punch press. This smaller hole caused a more uniform force to be measured as the catheter was inserted through it and contacted the sheet on its entire circumference (Figure 37). This piece of silicon sheeting was then placed between the first and second stacked test tube holders and the clamps were replaced to hold the material taught and centered on the measurement system.
Figure 36: Test set up for method three. Lateral view of the set up. This test method proved to be the most accurate by far of the three methods attempted for this friction measurement test.

Figure 37: Aerial view of the set up for test method 3 without catheter attached.

Results
Method 3 proved successful in providing consistent data for each catheter and little variation between catheters of the same coating as evidenced in Figures 38 and 39. Slight differences in force required for insertion were measured between coating methods and accurately account for the difference in surface which is readily described by the SEM images taken prior to this friction evaluation. The statistical analysis was run using GraphPad Prism software for a one-
way ANOVA test. The data of these trials are shown in Tables 8 and 9. All friction data can be
seen in Appendix d and complete statistical analysis can be seen in Appendix f. Overall, the
coated catheters proved much easier to insert than the uncoated catheters and demonstrate that a
synthetic spider silk coating decreases the friction involved in insertion.

Table 8: Statistical analysis of the average insertion force for each of the catheter coating
methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Average Force (N)</th>
<th>Standard Error of the Mean</th>
<th>Standard Deviation</th>
<th>Lower 95% Confidence Interval of Mean</th>
<th>Upper 95% Confidence Interval of Mean</th>
<th>Significant Compared to Uncoated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoated</td>
<td>3.764</td>
<td>0.06836</td>
<td>0.2051</td>
<td>3.606</td>
<td>3.922</td>
<td>No</td>
</tr>
<tr>
<td>Spray</td>
<td>0.73</td>
<td>0.05</td>
<td>0.15</td>
<td>0.62</td>
<td>0.85</td>
<td>Yes</td>
</tr>
<tr>
<td>Spray + Chlorhexidine</td>
<td>0.74</td>
<td>0.05</td>
<td>0.15</td>
<td>0.62</td>
<td>0.85</td>
<td>Yes</td>
</tr>
<tr>
<td>Dip</td>
<td>0.74</td>
<td>0.05</td>
<td>0.15</td>
<td>0.62</td>
<td>0.85</td>
<td>Yes</td>
</tr>
<tr>
<td>Dip + Chlorhexidine</td>
<td>1.15</td>
<td>0.16</td>
<td>0.47</td>
<td>0.78</td>
<td>1.51</td>
<td>Yes</td>
</tr>
<tr>
<td>Aerosolize</td>
<td>0.83</td>
<td>0.11</td>
<td>0.34</td>
<td>0.57</td>
<td>1.09</td>
<td>Yes</td>
</tr>
<tr>
<td>Aerosolize + Chlorhexidine</td>
<td>1.36</td>
<td>0.15</td>
<td>0.45</td>
<td>1.02</td>
<td>1.70</td>
<td>Yes</td>
</tr>
<tr>
<td>Dip and Aerosolize</td>
<td>1.30</td>
<td>0.23</td>
<td>0.69</td>
<td>0.77</td>
<td>1.83</td>
<td>Yes</td>
</tr>
<tr>
<td>Dip and Aerosolize + Chlorhexidine</td>
<td>1.02</td>
<td>0.15</td>
<td>0.46</td>
<td>0.63</td>
<td>1.38</td>
<td>Yes</td>
</tr>
<tr>
<td>Spray and Dip</td>
<td>1.34</td>
<td>0.31</td>
<td>0.93</td>
<td>0.63</td>
<td>2.06</td>
<td>Yes</td>
</tr>
<tr>
<td>Spray and Dip + Chlorhexidine</td>
<td>0.66</td>
<td>0.06</td>
<td>0.17</td>
<td>0.54</td>
<td>0.79</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Table 9: Statistical analysis of the maximum insertion force for each of the catheter coating methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Max Force (N)</th>
<th>Standard Error of the Mean</th>
<th>Standard Deviation</th>
<th>Lower 95% Confidence Interval of Mean</th>
<th>Upper 95% Confidence Interval of Mean</th>
<th>Significant Compared to Uncoated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoated</td>
<td>4.01</td>
<td>0.06</td>
<td>0.19</td>
<td>3.86</td>
<td>4.16</td>
<td>No</td>
</tr>
<tr>
<td>Spray</td>
<td>1.05</td>
<td>0.05</td>
<td>0.15</td>
<td>0.94</td>
<td>1.17</td>
<td>Yes</td>
</tr>
<tr>
<td>Spray + Chlorhexidine</td>
<td>1.05</td>
<td>0.05</td>
<td>0.15</td>
<td>0.94</td>
<td>1.17</td>
<td>Yes</td>
</tr>
<tr>
<td>Dip</td>
<td>1.73</td>
<td>0.24</td>
<td>0.71</td>
<td>1.18</td>
<td>2.27</td>
<td>Yes</td>
</tr>
<tr>
<td>Dip + Chlorhexidine</td>
<td>1.45</td>
<td>0.14</td>
<td>0.42</td>
<td>1.12</td>
<td>1.78</td>
<td>Yes</td>
</tr>
<tr>
<td>Aerosolize</td>
<td>1.27</td>
<td>0.09</td>
<td>0.28</td>
<td>1.05</td>
<td>1.48</td>
<td>Yes</td>
</tr>
<tr>
<td>Aerosolize + Chlorhexidine</td>
<td>1.81</td>
<td>0.14</td>
<td>0.41</td>
<td>1.49</td>
<td>2.13</td>
<td>Yes</td>
</tr>
<tr>
<td>Dip and Aerosolize</td>
<td>1.63</td>
<td>0.23</td>
<td>0.68</td>
<td>1.11</td>
<td>2.15</td>
<td>Yes</td>
</tr>
<tr>
<td>Dip and Aerosolize + Chlorhexidine</td>
<td>1.44</td>
<td>0.14</td>
<td>0.43</td>
<td>1.11</td>
<td>1.77</td>
<td>Yes</td>
</tr>
<tr>
<td>Spray and Dip</td>
<td>1.75</td>
<td>0.30</td>
<td>0.91</td>
<td>1.05</td>
<td>2.45</td>
<td>Yes</td>
</tr>
<tr>
<td>Spray and Dip + Chlorhexidine</td>
<td>1.05</td>
<td>0.07</td>
<td>0.21</td>
<td>0.89</td>
<td>1.21</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Average Insertion Force

Figure 38: Data shown are the mean insertion forces ± SEM for each coating method. Each number corresponds to the spider silk coating type on the project overview and the addition of “C” represents coatings containing chlorhexidine. Different letters indicate groups are statistically different (p < 0.05) according to ANOVA with Tukey HSD post-hoc tests (GraphPad Prism).
Figure 39: Data shown are the max insertion forces ± SEM for each coating method. Each number corresponds to the spider silk coating type on the project overview and the addition of “C” represents coatings containing chlorhexidine. Different letters indicate groups are statistically different (p < 0.05) according to ANOVA with Tukey HSD post-hoc tests (GraphPad Prism).

Conclusions
The results obtained from the third friction test method indicated the addition of all coatings to the Foley catheters decreased the force needed for insertion. Uncoated Foley catheters required approximately 4 N of force during insertion through the 9-mm diameter silicon hole, while coated catheters required less than 2 N forces. Several coating methods displayed statistical difference between insertion force values (Figure 38 and 39). A summary of all decisions made to design the friction test is shown in Figure 40.
Figure 40: Design tree detailing the design decisions developed for friction testing.
vi. Bend/Bunch Testing

Catheter insertion is an uncomfortable experience for all patients, but is especially uncomfortable for male patients. Due to the longer male urethra and the presence of a prostate, catheters must be flexible during insertion. It is critical that the coating on a catheter does not tear off when it is bent. Ideally, catheter coatings would stay consistent and would not crack during bending, but this is not critical to catheter function. Should a catheter coating crack and flake off of the catheter, this could contaminate any urine samples drawn from the catheter. However, cracking would not affect samples and would not compromise the function of the catheters. Furthermore, most elderly male patients have enlarged prostates which cause catheters to bunch at the end. The characteristics of the coating must be tested when the catheters are bunched at the end. To test the coating characteristics under bend/bunch conditions, a testing fixture was 3D printed to hold catheters in a bent position while they were imaged.

Materials

- AmScope Light Microscope
- Onshape.com Online CAD program
- 3D printer
- Coated catheters

Methods

First, a bunch test fixture was designed using SolidWorks. The idea of the first iteration of the part was to place catheters inside a curved tunnel for 3 hours and image after removal using light microscopy. The design drawing is shown in Figure 41. The first part iteration was designed with holes in arcs with 1, 2, 4, 8, and 16-inch diameters.

With this experimental design, however, the catheters were imaged in a straightened position, minimizing the ability to view cracking and flaking. Because coating visualization was far easier when catheters were bent, a new fixture design was necessary. Furthermore, the 1-inch and 2-inch diameter holes were removed after examining catheter usage in clinical settings. Catheters will never be bent to that angle without becoming bunched. Therefore, the bunching portion of the test would cover these diameters. The drawing of the final iteration of the testing fixture is shown in Figure 42.

The second testing fixture allowed images to be taken while the catheters were held in a bent position. Catheters were placed in each arch and imaged using the AmScope light microscope at 5X magnification, starting with the 16-inch arc.
Figure 41: The CAD drawing of the first iteration of the bend test fixture. Five different diameter hollows were designed for coated catheters to be inserted through. The smallest two diameter hollows were deemed unnecessary because urinary catheters are not exposed to such extreme bending in actual practice.

Figure 42: Drawing of final bend test fixture. The top surface was removed in this design to allow easier placement of catheters within the three hollows. Width of the hollows was increased so the coating of the catheters was not removed upon placement. Overall dimensions were minimized to decrease printing expenses.
Results
The results of the 50.4 mm diameter arc and bunch tests are shown in Figures 43 and 44. All images are available in Appendix c. Note the cracking visible in most coatings when bunched. Though the coatings did crack, none flaked off the catheter. A summary of all results is given in Table 10.

Figure 43: Catheters placed in the 8-inch diameter arc. All images on the left are without chlorhexidine. (1) Spin (2) Dip (3) Aerosolized (4) Dip and aerosolized, (5) Spin and dip. Note the cracking visible in images (2), (4), and (5).

Figure 44: Catheters bunched at tip. All images on the left are without chlorhexidine. (1) Spin (2) Dip (3) Aerosolized (4) Dip and aerosolized, (5) Spin and dip. Note the cracking visible in images (1), (2), (4), (5).
Table 10: Summary of Bend/Bunch test results.

<table>
<thead>
<tr>
<th>Coating Type</th>
<th>8 inch arc</th>
<th>Bunch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crack?</td>
<td>Flake?</td>
</tr>
<tr>
<td>Spin</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Spin + Chlorhexidine</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Dip</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Dip + Chlorhexidine</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Aerosolized</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Aerosolized + Chlorhexidine</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Dip and Aerosolized</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Dip and Aerosolized + Chlorhexidine</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Spin and Dip</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Spin and Dip + Chlorhexidine</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

**Conclusions**

As shown in the figures above, all coating methods except for the aerosolized coating cracked during bunch testing. Because the catheters only cracked and did not flake off, the coatings would not affect any diagnostic samples taken from the catheter in clinical use. Each of the coating methods was determined to be a viable method based on the bend/bunch testing parameters.

**vii. AFM Imaging**

**Materials**
- Atomic Force Microscope (AFM)
- Glass Microscope Slides
- Double Sided Tape
- Coated and uncoated catheters

**Methods**

A section of catheter was cut into a 10-cm strip. The bottom of the strip was uniform to avoid any bumps or inconsistencies that would prevent the AFM from obtaining a clear image. The strip was then secured to a glass microscope slide using double sided tape and placed under the cantilever of the AFM. The AFM was set to tapping mode and the cantilever was lowered onto the sample until contact was made. The AFM measured the topography of an area between 2500 and 10000 µm² and a 2D image was created. Three images of each catheter were taken. Once the image was taken, the mean roughness of each catheter was measured using the AFM software and 3D images were created for several of the catheters.

**Results**

All 2D AFM images can be found in Appendix e. A 3D image of an uncoated catheter is shown in Figure 45. A 3D image of a dip and aerosolize coated catheter with chlorhexidine is shown in Figure 46. A 3D image of a dip coated catheter with chlorhexidine is shown in Figure 47. These images are shown for their high quality and the consistency across coating types. The mean roughness measurements of the catheter images are shown in Table 11. There are large
differences between images of the same catheter due to micro-imperfections in the coatings such as cracks.

Figure 45: 3D image of the topography of an uncoated catheter. The scan size in the horizontal directions is 50 μm and the data scale in the vertical direction is 3 μm.

Figure 46: 3D image of the topography of a dip and aerosolize coated spider silk and chlorhexidine catheter. The scan size in the horizontal directions is 50 μm and the data scale in the vertical direction is 3 μm.
Figure 47: 3D image of the topography of a dip coated spider silk and chlorhexidine catheter. The scan size in the horizontal directions is 50 µm and the data scale in the vertical direction is 3 µm.

Table 11: The mean roughness of catheters calculated from AFM images.

<table>
<thead>
<tr>
<th>Coating</th>
<th>Image 1 (nm)</th>
<th>Image 2 (nm)</th>
<th>Image 3 (nm)</th>
<th>Average (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoated</td>
<td>73.005</td>
<td>61.453</td>
<td>63.65</td>
<td>66.036</td>
</tr>
<tr>
<td>Dip</td>
<td>131.74</td>
<td>244.22</td>
<td>88.15</td>
<td>154.703</td>
</tr>
<tr>
<td>Dip with Chlorhexidine</td>
<td>88.332</td>
<td>45.316</td>
<td>68.841</td>
<td>67.496</td>
</tr>
<tr>
<td>Aerosolized</td>
<td>146.76</td>
<td>76.845</td>
<td>375.52</td>
<td>199.708</td>
</tr>
<tr>
<td>Aerosolized with Chlorhexidine</td>
<td>102.62</td>
<td>107.8</td>
<td>550.7</td>
<td>253.706</td>
</tr>
<tr>
<td>Spray</td>
<td>192.71</td>
<td>221.95</td>
<td>315.36</td>
<td>243.34</td>
</tr>
<tr>
<td>Spray with Chlorhexidine</td>
<td>248.18</td>
<td>86.586</td>
<td>99.953</td>
<td>144.906</td>
</tr>
<tr>
<td>Dip &amp; Aerosolize</td>
<td>29.053</td>
<td>26.633</td>
<td>46.694</td>
<td>34.127</td>
</tr>
<tr>
<td>Dip &amp; Aerosolize with Chlorhexidine</td>
<td>73.208</td>
<td>191.04</td>
<td>163.53</td>
<td>142.593</td>
</tr>
<tr>
<td>Dip &amp; Spray</td>
<td>76.279</td>
<td>113.78</td>
<td>44.097</td>
<td>78.052</td>
</tr>
<tr>
<td>Dip &amp; Spray with Chlorhexidine</td>
<td>73.164</td>
<td>168.95</td>
<td>271.59</td>
<td>171.235</td>
</tr>
</tbody>
</table>

Conclusions
Several divots can be seen in the image of the uncoated catheter. Divots are an optimal location for bacterial colonization, increasing the risk of CAUTIs. Both 3D images of spider silk coated catheters show smooth surfaces with no divots. Though several bumps can be seen on the surfaces, they are not optimal locations for bacterial colonization due to their smoothness. The crack seen in Figure 47 is similar to cracks seen in many other images and is likely a gap between different drops of spider silk. This could be a possible attachment point for bacteria, though the antimicrobial properties of the coating would inhibit bacterial growth in these areas. These images show the relative smoothness of coated catheters.
viii. SEM Imaging

Materials
- Scanning Electron Microscope (SEM)
- SEM sample mount
- Coated catheters

Methods

Catheter samples of each coating method were cut transversely to an approximate thickness of 1 cm, secured to an SEM sample mount, and placed in the machine. Imaging was carried out in the SEM under vacuum. FenAnn Shen operated the instrument with all group members present to help direct the process.

Results

Several representative SEM images displaying the measured thickness of the synthetic spider silk coating are shown in Figures 48 through 55. Appendix e contains all SEM images taken. The bar at the bottom of each image shows the conditions used during imaging including the magnification. A scale bar is also shown for reference.

Figure 48: SEM image of a spray coated catheter. The SEM software was used to measure the thickness of the coating (1.133 µm).
Figure 49: SEM image of a dip coated catheter. The SEM software was used to measure the thickness of the coating (1.759 µm) and the distance from the catheter to the top of the flake (4.049 µm) that was caused by cutting with a razor blade.

Figure 50: SEM image of a dip coated catheter + chlorhexidine. The SEM software was used to measure the thickness of the coating (1.511 µm).
Figure 51: SEM image of aerosolize coated catheter. The SEM software was used to measure the thickness of the coating (1.027 µm).

Figure 52: SEM image of dip and aerosolize coated catheter. The SEM software was used to measure the thickness of the coating (1.512 µm).
Figure 53: SEM image of spray and dip coated catheter. The SEM software was used to measure the thickness of the coating (2.952 µm).

Figure 54: SEM image of aerosolize inside catheter coating. The SEM software was used to measure the thickness of three different coating beads. From left to right: 20.25 µm, 14.84 µm, and 12.14 µm.
Figure 55: SEM image of dip inside catheter coating. The SEM software was used to measure the thickness of a coating bead (1.769 µm).

Conclusions
The SEM images that were obtained provided qualitative results to whether the applied coatings were smooth. Dip, dip and aerosolize, and spray and dip all showed very smooth surfaces. The spray and aerosolize methods appeared much rougher. The catheters were difficult to cut due to the latex material that they are made of. This resulted in the appearance of flaking in some of the SEM images.

SEM images is very time consuming so all of the catheter samples that were imaged were synthetic spider silk coating without chlorhexidine. FenAnn Shen assured the group that the addition of chlorhexidine would not change the SEM pictures in any way. However, in order to be sure of this, the team decided to image a dip coated catheter and a dip coated catheter with chlorhexidine in order to ensure that the coatings were similar (Figures 49 and 50 respectively). The resulting thicknesses were 1.759 µm and 1.511 µm respectively however, no other differences were noted. This confirmed that imaging only the synthetic spider silk coated catheters was sufficient.

Industry leaders expressed interest in a catheter that is coated on the inside as well. As mentioned in the coating protocols above, the team developed an aerosolized inside coating method and dip inside coating method to meet this interest. However, the SEM images revealed that neither method created an even coating (Figures 54 and 55) since there was no way to control the flow of spider silk through the catheter in either method. Due to time constraints, the team decided to stop investigating methods to create an inner coating.
7. Final Design Review

A summary table (Table 12) was created evaluating the various catheter coating methods based on the design criteria: antimicrobial activity, insertion friction, surface roughness, coating flexibility, and production feasibility. For antimicrobial activity, coatings that had statistically significant zones of inhibition when compared to uncoated catheters were given one point for each bacteria species (total of three). Coating methods that significantly reduced friction when compared to an uncoated catheter were given a score of one. Roughness was given a qualitative score based on SEM imaging: a smooth appearance received a one while a rough appearance received a zero. The coating flexibility was based on the bend bunch test. Coating methods that did not crack received a one, and coatings that did crack scored zero. The feasibility scores were based on the team’s opinion on ease of manufacturing. A score of two is ease of manufacturing compared to the current process of creating urinary catheters; a score of one is harder to manufacture than the current process, and a score of zero is a non-feasible coating for manufacturing. Based on this scoring process, the best coating methods were dip with chlorhexidine, spray and dip with chlorhexidine, and dip and aerosolized with chlorhexidine.

Table 12: Summary of Evaluation Criteria

<table>
<thead>
<tr>
<th>Uncoated</th>
<th>Dip</th>
<th>Dip with Chlorhexidine</th>
<th>Spray</th>
<th>Spray with Chlorhexidine</th>
<th>Aerosolized with Chlorhexidine</th>
<th>Spray and Dip with Chlorhexidine</th>
<th>Dip and Aerosolized with Chlorhexidine</th>
<th>Dip and Aerosolized with Chlorhexidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimicrobial:</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Friction:</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Roughness:</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Bend/Bunch:</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Ease:</td>
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<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total:</td>
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<td>3</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

8. Conclusions

Multiple methods for coating of urinary catheters using synthetic spider silk were developed. However, results showed that only three coating methods exhibited significant antimicrobial properties. All methods were scored on their antimicrobial properties, insertion force requirements, surface roughness, ability to resist flaking when bent and bunched, and mass manufacturing feasibility. Two coating methods tied for the highest score: the dip with chlorhexidine and dip and aerosolized with chlorhexidine. However, because the feasibility score is based on qualitative results, the team recommends the dip and aerosolized with chlorhexidine coating as the best catheter coating to reduce the risk of CAUTIs.

Catheters coated using this method met all of our design objectives and, in particular, showed statistically significant larger bacterial zones of inhibition against three UTI-causing bacterial strains. This was the most important objective for the product because significant antimicrobial properties will reduce the possibility of CAUTIs. It was further determined that this coating requires a smaller force to insert through a synthetic urethra analog which means that the coating is smoother than uncoated Foley catheters. The coating did not flake off when the catheter was bent or bunched at the tip which is critical. Flaking of the coating would cause the catheter to lose antimicrobial properties. This coating would require additional work to upscale to mass
manufacturing levels when compared to the process of manufacturing uncoated catheters. Based on the antimicrobial and mechanical success of this coating the team recommend the dip and aerosolized with chlorhexidine coated catheter as the final product.

9. Recommendations for future work

Though the design of an antimicrobial Foley catheter was successful, there are many recommendations for future work. Testing with biological fluids such as urine is important to determine if there is any effect on the coating. It would also be important to compare the antimicrobial and mechanical results of the final product with those of silver-coated catheters. A cost analysis comparing the two products would also be beneficial to determine if the synthetic spider silk coated catheter would be successful in the current market.

An additional test for surface fouling due to the adsorption of proteins such as fibrinogen should also be performed. Fibrinogen is involved in blood coagulation which is a concern during catheter use. Additionally, cytotoxicity assays should be completed to determine host cell response to the presence of the coated catheters.

There are several changes that could be made to the coating protocols outlined in this design report. Inner surface catheter coatings were examined but were not successful. However, industry leaders remain interested in inner coatings of catheters. Therefore, new methods could be developed based on the design completed in this project to successfully coat the inside of urinary catheters. The effect of nanoparticles on antimicrobial properties when included in the synthetic spider silk solutions before coating could be investigated as well.
Reflection

My senior capstone project was the biggest project that I have worked on during my time as an undergraduate student at USU. Not only was it the biggest project, but the most difficult and time consuming as well. The project started with a concept that was introduced in our senior design class at the beginning of the year and ended with a synthetic spider silk coated urinary catheter that was created by me and six of my fellow biological engineering students.

The project started with a lot of brainstorming and literature review. We had an idea of what we wanted the product to be but we did not have knowledge of the catheter industry and the products that already exist. A previous group from Dr. Lewis’ lab preformed research on synthetic spider silk coatings on intravenous catheters (IV). We sought to expand their research and apply it to our urinary catheters.

We had to optimize coating protocols before we could start any of the coatings. The spray, dip, and spray and dip methods were used previously for IV coatings. The general methods were the same but we had to make slight modifications for urinary catheters. The previous group also expressed interest in trying an aerosolized coating method with the use of a nebulizer but were unable to coat their IV catheters. We included the aerosolized method and also developed the dip and aerosolized method. Both methods required new coating protocols that were developed by the team. Once all of the methods were optimized we were able to coat all the catheters and start antimicrobial and mechanical testing.

This project required that we use skills that we have learned throughout our education at USU. It was amazing to see things that we had learned be applied in a practical way. Antimicrobial testing was completed by performing zone of inhibition testing which is something we learned about and practiced in our required microbiology lab. The mechanical testing we performed with the MTS Instron required use of an instrument that we’ve been taught to use many times by engineering professors over the last four years. The bend/bunch testing that we did required a 3D printed part that was made with SolidWorks which is a program that a few of us learned in an engineering graphics class. The statistics that we used to evaluate our final product were taught to us in our statistics class. My proudest moment was at the end of the project when I created the poster that we used to present our final product. I have worked in Dr. Benninghoff’s research lab on campus for almost three years and have had many presentation opportunities. I have learned how to make research posters under the guidance of both Dr. Benninghoff and Ph.D student Sumira Phatak. Creating the poster was probably the least stressful part of this design project because of all of my experience. When I first started working in Dr. Benninghoff’s lab I was terrified of presenting because I didn’t know how to make a poster and I didn’t like public speaking. Presentations are now my favorite part of a research project because I enjoy getting the opportunity to talk to others about something that I am passionate about. This project has shown me how well all of my research and education opportunities at USU have prepared me for my future. I am very grateful for all of the professors and fellow classmates who have helped me along the way.
We had mentors to help us through this design process but both Dr. Taylor and Dr. Lewis maintained a hands-off approach. They were very helpful and always willing to offer advice but they left us to brainstorm and solve problems as they occurred. I really appreciated that they wanted us to do the work on our own because when I find a job after I graduate I will be expected to accomplish tasks on my own. I was treated like a professional which was very motivating because I wanted to prove that I am a self-sufficient engineer.

I learned a lot during this capstone design project about myself and the people that I worked with. This past year has been extremely stressful and, although I’ve learned how to successfully complete such a big project, I am glad to be finished and I’m proud of the work I’ve done. There are many lessons I’ve learned and I want to share them with anyone working on a capstone project in the future.

1. Communication is key - My design team had a total of seven members and I learned very quickly that it is almost impossible for everyone to be on the same page. One of my biggest regrets is that we did not settle on communication expectations at the beginning of the project and our team dynamic suffered greatly because of it. Find a form of communication that works for everyone. We tried text messages, emails, and messaging apps but the only thing that seemed to work was a phone call when we needed to contact each other. Make sure that everyone on the team has the contact information for everyone in the group. I found out almost six months into the project that one of my team members did not have any of my contact information so he was not receiving any of the updates that I sent out. Team members are able to avoid duties that they don’t want to do if they don’t have constant communication with the rest of the team. It is important to keep each other accountable and let others know deadlines are approaching.

2. Don’t be afraid to lead – When we first started our design project I thought we would all be able to share duties and get things done in a timely manner. Unfortunately, that is not always the case. Everyone has a different schedule and meeting times might not always include all of the team members. This means that leaders are necessary in order to make sure that tasks get done. I was already good friends with some of my design team and thought of them as my equal so I felt very uncomfortable handing out assignments. However, the project will not be finished if everybody avoids the leadership role. I recommending rotating the leadership position so that everyone gets a chance to experience the expectations and the stress does not fall solely upon one person.

3. Start early – I was warned about time management by last years’ senior design teams but I don’t think our team took it very seriously. I hope that by passing on this advice maybe one year will get it right. We started working on our project in about March of 2017 and the final project was due December 2017. That means that we had almost nine months to work on our project. This may seem like a lot of time but with the addition of classes and other extra-curricular activities during the school year time will fly by. Make concrete deadlines and stick to them. Communicate with each other when there are reasons that those deadlines are unable to be met.
4. Ask questions – This project was difficult because it was a student-run project. Our design professor treated us like working engineers and expected us to finish the project on our own. I have taken many design classes over the last four years but there is still so much I can learn about both design and engineering. This can make the project seem very intimidating because there are so many unknown problems that you will have to find solutions to. I learned very quickly to ask for help when I needed it. If there is an instrument or procedure that you are not familiar with ask someone who does. Your professors have much more experience than you do and they are always willing to offer advice. If you are working on your project in a research lab there are always other undergraduate, graduate, and post-doctoral researchers around. They are a great resource especially if you are not familiar with the lab you are working in. Your team members are also an important resource. Everyone has different interests and backgrounds and someone on your team might have more experience with certain things.

5. Take chances – Our final product was a result of a conversation between me and a few of my teammates. We were all working on different aspects of the project and thought it would be a great idea to combine our ideas. It was not one of our original coating methods and we came up with the idea almost half-way through our project. Inspiration can strike at any point in your project. Don't discount an idea because it doesn't fit into your timeline or come at the beginning of your brainstorming process. Be open to trying new things.

Capstone projects are a great way to spend time on something that you are passionate about. I hope that I have offered insight into what the process is like and have provided helpful advice. Every project is different and you will run into problems along the way. But, if you work hard, the final project will be something that you are unbelievably proud of. That is the best feeling in the world.
10. References


