In this project the goal was to produce and analyze silk fibroin films. Silk is a fibrous protein which is made by the silkworm and spiders. There are two main proteins which compose silk: fibroin and sericin. The fibroin makes up the core of the silk fiber with the sericin surrounding it. Fibroin is responsible for silk’s strength while sericin is responsible for the structural integrity of individual silk fibers.

Silk fibroin is highly biocompatible with the human body, making it a good material to use for tissue engineering, wound dressings, drug release material, and even sensors. Electrospun fibroin nanofibers can be formed into a mat onto which cells can be seeded. A silk mat with growth factors or skin cells incorporated would be a good candidate for a wound dressing as it has nutrients and healthy cells which will promote quick healing. Silk films have seen uses in humidity sensors that can detect changes in respiration as well as in novel controlled drug release materials.

However, in order to create silk biomaterials for specific applications, one must understand how the handling and treatment of the protein will affect its structure. Structure impacts function. In silk the β-sheet secondary structure is responsible for the material’s water solubility. A higher β-sheet content will result in a less soluble film while fewer β-sheets will make a more soluble film. It is known that the method of water annealing can increase β-sheets in silk fibroin. Understanding the relationship between annealing time and β-sheet content will make it possible to fabricate silk materials with precise degradation times. To study this silk films were produced and subjected to water annealing for different amounts of time. Attenuated Total Reflection Fourier Transform infrared spectrometry (ATR-FTIR), was then used to analyze the changes in β-sheet content.

For this project silk from *Bombyx mori* silkworms was used. The tough cocoons are cut into pieces and degummed in a slightly basic solution. This removes the sericin and leaves fibroin fibers. The fibers are dissolved in a lithium bromide solution. This solution is then dialyzed against deionized water to make an aqueous silk solution. This solution is pipetted in 0.5 mL aliquots onto a hydrophobic surface and allowed to air dry, producing films (Figure 1). After the films are dry, they are stored in a container. To water anneal a film is placed in a vacuum chamber with a dish of deionized water. The vacuum is drawn to -30 mmHg and the film is allowed to anneal at room temperature for either 1, 3 or 6 hours. Once annealing is done the film is allowed to air dry. Then ATR-FTIR is done to analyze the structure of the film.

As can be seen in Figure 1 the silk films are transparent and reflective. The concentration of the silk solution affects the physical properties of the resulting film. A 1.8% silk solution will produce transparent films which are brittle and thin like cellophane. An 8% silk solution will produce films which are more robust and curved. These films are slightly flexible but will break if bent too far. Figure 2 shows the ATR-FTIR results. Two untreated films were subjected to each annealing time ie two films underwent water annealing for one hour. This was done to ensure consistency. The peak around 1620 cm\(^{-1}\) is characteristic of the β-sheet secondary structure in proteins. The peak around 1645 cm\(^{-1}\) is characteristic of random coils. It can be seen that by increasing the length of the water annealing the secondary structure of the silk is changed; more β-sheet is formed, and the peak around 1620 cm\(^{-1}\) becomes more intense. Water annealing forms β-sheets because the water molecules act as a plasticizer, lowering the glass transition temperature of the fibroin. The normally non-crystalline areas of the fibroin become more flexible allowing for more molecular movement, and ultimately form β-sheets. It would therefore make sense that a longer water annealing time leads to more β-sheet formation as the water has a longer time to plasticize the fibroin.

This is an important relationship to understand as one of the major advantages to using silk fibroin is its tunability and the consistency with which one can produce materials that have the same properties. This could result in silk biomaterials becoming mass produced and commercially available. As a future direction it would be interesting to attempt to incorporate another material into the silk solution before the film is cast. Graphene could be dissolved into the silk solution and make the resulting film conductive. This might have use as a biosensor. However, adding another material into the film will likely alter the physical characteristics of it, possibly making it more fragile or the film retrieval process more difficult.