SYNTHESIS AND CHARACTERIZATION OF NATURALLY-DERIVED SILK FIBROIN FIBERS AND FILMS FOR THE DESIGN OF NON-METALLIC TUNABLE AND TISSUE RESPONSIVE FIXATION DEVICES IN REGENERATIVE SURGERY

Neveatha Muthusamy, Biomedical Engineering, SBHSE

Mentors: Vincent Pizziconi, Ph.D, SBHSE; Erwin Kruger M.D, Mayo Clinic

INTRODUCTION

Polymers are widely used for bone tissue engineering but none have been used for bone fixation. Only silk fibroin is being considered due to its superior mechanical properties among polymers. Natural silk has attracted much interests by both the biomaterials and regenerative medicine communities due to its physical and chemical properties with its enhanced mechanical strength and enzymatic degradation which is the key design factors for hard tissue regeneration and repair. Silk fibroin (SF) is derived from cocoons produced by silkworms which contains a fibroin inner core and a sericin outer shell.

OBJECTIVE

The objective of this work is to synthesize and characterize the mechanical and chemical properties of the natural biopolymer, silk fibroin which will be used to further assess the utility of SF as a biomaterial replacement of rigid, metallic surgical hardware.

METHODS & MATERIALS

Degumming Process: Cocoons derived from *Bombyx Silk* were boiled at 100ºC added with 0.02M of sodium carbonate for 30 minutes, then squeezed the fibers and dried overnight.

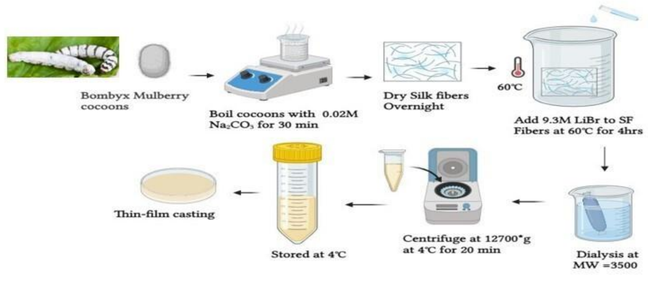


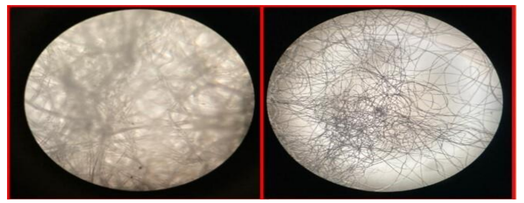
Fig 1: Process of SF Production

Dissolving the fibers: The dried cocoons were added with 13.8g (9.3M) of lithium bromide (libr) to dissolve the dried fibers at 60ºC for 4hrs and dialysis was carried out for 48hrs followed by centrifugation, twice at 12,700*g at 4ºC for 20 minutes; sediment was removed using a 100µm nylon membrane and filtered SF solution was stored at 4ºC.

RESULTS

Verification by Weight Loss %: After degumming, a weight loss of 34% was determined which is consistent with the 34% composition of sericin in silk fibers.

Optical Characterization: Optical images of silk fibers from cocoons (Fig 2a) and degummed silk fibers (Fig 2b), show gross morphological differences, as expected (4X) .



[a] **[b]**

Fig 2: Optical Microscopy Silk Fibroin Fibers (4X)

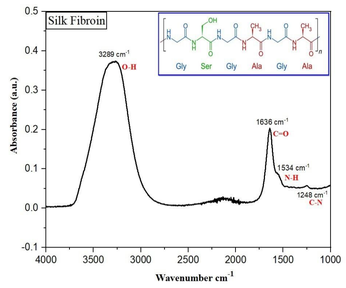


Fig 3: FTIR Characterisation

FTIR Characterization: FTIR spectra obtained from SF polymer solution indicated a strong, broad peak at 3289 cm^{-1} due to O-H bonding. In addition, the peaks shown at 1636 cm^{-1}, 1534 cm^{-1} and 1248 cm^{-1} are the corresponding peaks of C-O (amide I), N-H (amide II), and C-N (amide III) associated with the characteristic β-sheet of silk fibroin.

Thin Film Casting: The SF polymer solution produced from cocoons were used to make a thin SF films using the solvent cast method. 2ml of SF solution is poured over the petri dish and dried vernight, where the water molecules vaporated and formed a thin layer of SF ilms shown in Fig 5.

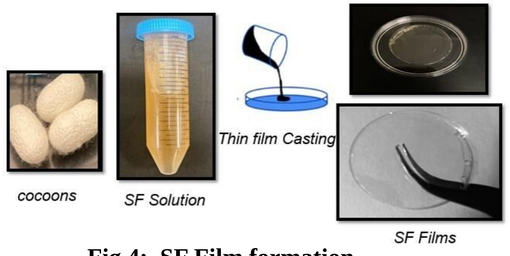


Fig 4: SF Film formation

SF Mechanical Properties: The mechanical properties of degummed SF fibers were measured using an Instron mechanical test system. Both tensile strength and elastic modulus were derived from the stress-strain curve shown in Figure 5. Tensile strength was estimated to be 350MPa-400MPa and the elastic modulus was determined to be 6-8GPa.

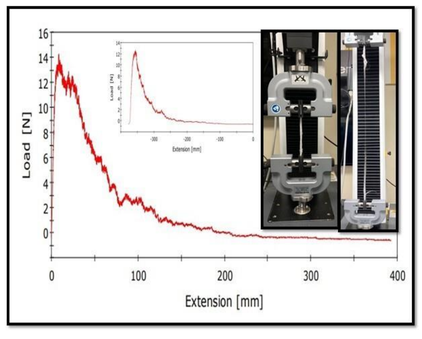


Fig 5. Stress-strain Response SF Fibers

CONCLUSION

- ✓ Silk fibroin polymer solution was successfully synthesized as confirmed by weight loss calculation, morphological differences observed from optical microscopy and FTIR.
- ✓ Data obtained from mechanical tests performed on degummed silk fibroin fibers provided values of tensile strength and elastic modulus that were consistent with published values.
- ✓ Silk fibroin films, successfully made from the solvent cast process, are of sufficient quality to carry out the silk fibroin degradation studies aimed at assessing the tunability of non-metallic, SF polymer-composite fixation devices for next generation regenerative surgery applications.

NEXT STEPS

Degradation Studies: Vmax (maximum velocity) and Km (Michaelis-Menten constant) are the two important experimental parameters to describe the kinetics of an enzyme catalysed reaction as described in the Michaelis-Menten rate equation for a substrate enzyme-catalyzed reaction (Equation 1 below). Using the appropriate values of Vmax and Km for each for the enzymes, α-chymotrypsin and protease XIV, the degradation rates of silk fibroin cast film substrates will be determined.

$$v = \frac{V_{max}[S]}{K_M + [S]} \quad (1)$$

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*[2] Diane Umuhoza, Fang Yang, Dingpei Long, Zhanzhang Hao, Jing Dai,and Aichun Zhao "Strategies for Tuning the Biodegradation of Silk Fibroin-Based Materials for Tissue Engineering Applications", ACS Biomaterials Science & Engineering **2020** 6 (3), 1290-1310, DOI: 10.1021/acsbiomaterials.9b01781.*

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