Optimization of Silk Fibroin Processing Conditions for Electrospinning in Biomedical Applications

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Background

Silk fibroin (SF), a structural protein from *Bombyx mori* silkworm cocoons, is a **promising biomaterial** for regenerative medicine due to its:

- Biocompatibility
- Mechanical Strength
- Tunable Degradability
- Potential for Genetic Editing

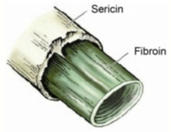

Fig. 1. Protein Structure of B. mori silk fiber

A key challenge in SF processing is **premature gelation**, which prevents consistent electrospinning and limits reproducibility in scaffold fabrication. Understanding the factors that drive gelation is critical for producing stable, high-quality SF solutions.

By refining processing parameters, this study supports the fabrication of **biocompatible, non-metallic load-bearing implants**, such as the silk-based bone screw being developed by the BioICAS Lab in collaboration with the Mayo Clinic, advancing safer and more sustainable materials for regenerative medicine.

Silk Fibroin Gelation

Gelation occurs when soluble silk fibroin molecules self-assemble into a solid or semi-solid network through **β-sheet formation**, driven by **hydrogen bonding** and **hydrophobic interactions** between protein chains.

Environmental factors such as **pH, temperature, salts, shear stress, and fibroin concentration** strongly influence this transition.

Research Aims

The research aims of this project are:

- Determine chemical degumming process factor(s) responsible for Silk Fibroin gelation
- Develop a robust Standard Operation Procedure (SOP) for the Extraction of Silk Fibroin from Silk Cocoons
- Demonstrate Reliability of Routinely Extracting Silk Fibroin from Silk Cocoons
- Validate Integrity of Silk Fibroin Using FTIR Analyses for determination of Key Molecular Bond Structures

Experimental Methods

1. B. mori Silk Fibroin Extraction Process

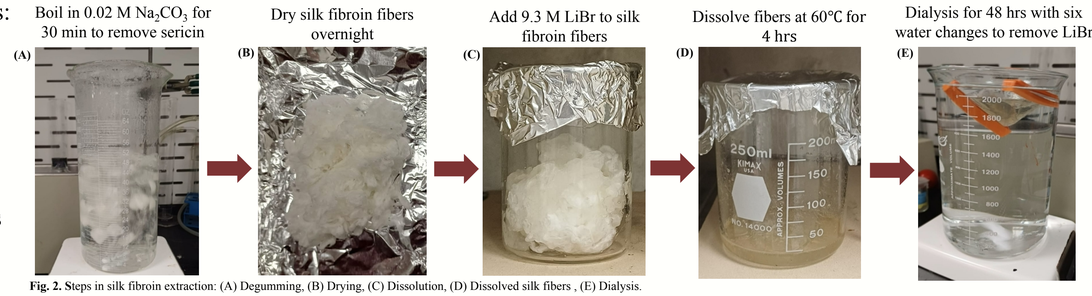

Fig. 2. Steps in silk fibroin extraction: (A) Degumming, (B) Drying, (C) Dissolution, (D) Dissolved silk fibers , (E) Dialysis.

2. Characterization of Silk Fibroin

Fourier Transform Infrared Spectroscopy (FTIR), was used to confirm silk fibroin structure and integrity.

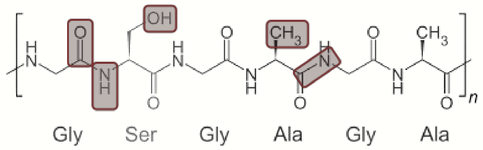

Fig. 3. Key functional groups and bonds in silk fibroin

Key Functional Groups / FTIR Bands for SF:

- C=O (Amide I): ~1620 - 1670 cm^{-1}
- N–H bend + C–N stretch (Amide II) ~1530 - 1560 cm^{-1}
- C–N stretch + N–H bend (Amide III) ~ 1220 - 1275 cm^{-1}
- N–H (Amide A): ~3270 - 3300 cm^{-1}
- N–H (Amide B): ~ 3060 - 3100 cm^{-1}
- O–H (Ser): ~3200 - 3600 cm^{-1}
- C–H (Ala): ~ 2850 - 2950 cm^{-1}

Results

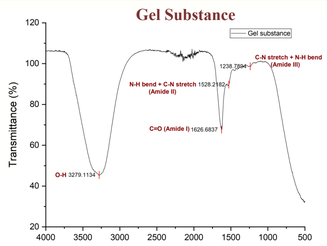

Fig. 4. FTIR of gel produced in initial extraction (stored LiBr)

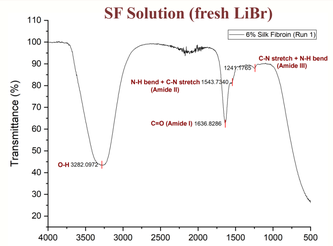

Fig. 6. FTIR of silk fibroin solution (fresh LiBr)

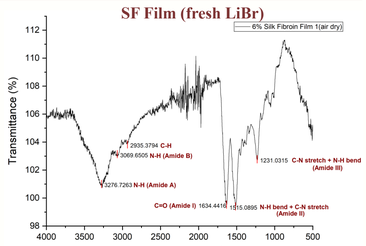

Fig. 8. FTIR of silk fibroin films (fresh LiBr)

Stored LiBr: silk fibroin solution gelled during dialysis

Fig. 5. Gel substance formed during dialysis with stored LiBr.

Fig. 7. SF solution extracted form B. mori cocoons

Fresh LiBr: silk fibroin remained in solution form

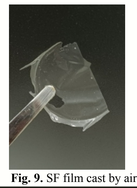

Fig. 9. SF film cast by air drying SF solution

Extracted silk fibroin solution was cast into films by air drying and heating in oven at 30°C

Discussion

Gelation behavior: Slight variations in processing strongly influenced fibroin solubility. Solutions made with stored LiBr gelled prematurely, while fresh LiBr produced stable, clear solutions.

FTIR confirmation: Characteristic amide I, II, and III peaks confirmed silk fibroin integrity. C–H bands (2850–2950 cm^{-1}) were faint, likely masked by water. The gel was verified as silk fibroin, though not in a structure suitable for electrospinning.

Key takeaway: Fresh LiBr and consistent handling improve fibroin solubility, reproducibility, and overall solution quality, which is critical for electrospinning applications.

Outcome: A detailed standard operating procedure (SOP) for *Bombyx mori* silk fibroin extraction was prepared, enhancing reproducibility and reliability across future experiments.

Future Work

1. Run SF extraction process based on detailed SOP that can be statistically analyzed (run in triplicates)
2. Continue testing the impact of temperature and pH on fibroin extraction
3. Apply optimized fibroin solutions to fabricate biofunctional scaffolds incorporating RGD peptides for regenerative applications

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