

Sericin as a Novel Biomaterial for Use in Wound Healing



Aditi Rao, BSE Biomedical Engineering (Biological Devices)
Mentors: Dr. Vincent Pizziconi, Dr. Erwin A. Kruger
School of Biological and Health Systems Engineering (SBHSE)

Introduction

Silk is a biomaterial that is composed of two proteins: silk fibroin (inner) and sericin (outer). Despite a similar composition of amino acids in both proteins, sericin is continually discarded as a by-product of the degumming process.

In recent times, silk has attained widespread attention as a bioinspired material due to:

- High tensile & mechanical strength
- Regenerative biocompatibility
- Optimal degradation properties



Fig 1. Silk scaffold

This research is focused on developing a sericin electrospun scaffold to investigate its biocompatibility using ISO 10993-5 MTT Cytotoxicity cell assay and to understand its suitability as a biomimetic substitute for extracellular matrix (ECM) tissue regeneration, followed by tunable degradation of the sericin scaffold. The proposed research aligns with Dr. P's BioICAS lab to develop bioactive polymer-ceramic nanocomposite surgical hardware and Mayo Clinic's plastic hand surgeon, Dr. K's interest in translating this advanced tunable Bioware for regenerative surgery.

Research Aims

The specific aims of this research efforts are:

1. Extract sericin from silk fibers, obtained from domestically farmed, lab-grown *Bombyx mori* silkworm cocoons, using the appropriate degumming process.
2. Design and implement an in vitro test method to investigate the feasibility of incorporating an enzyme-based, tunable process for the temporal control of the degradation of a sericin scaffold utilized for wound healing



Fig 2. Protein components of silk

Methods

Degumming is a process that isolates the proteins of silk.

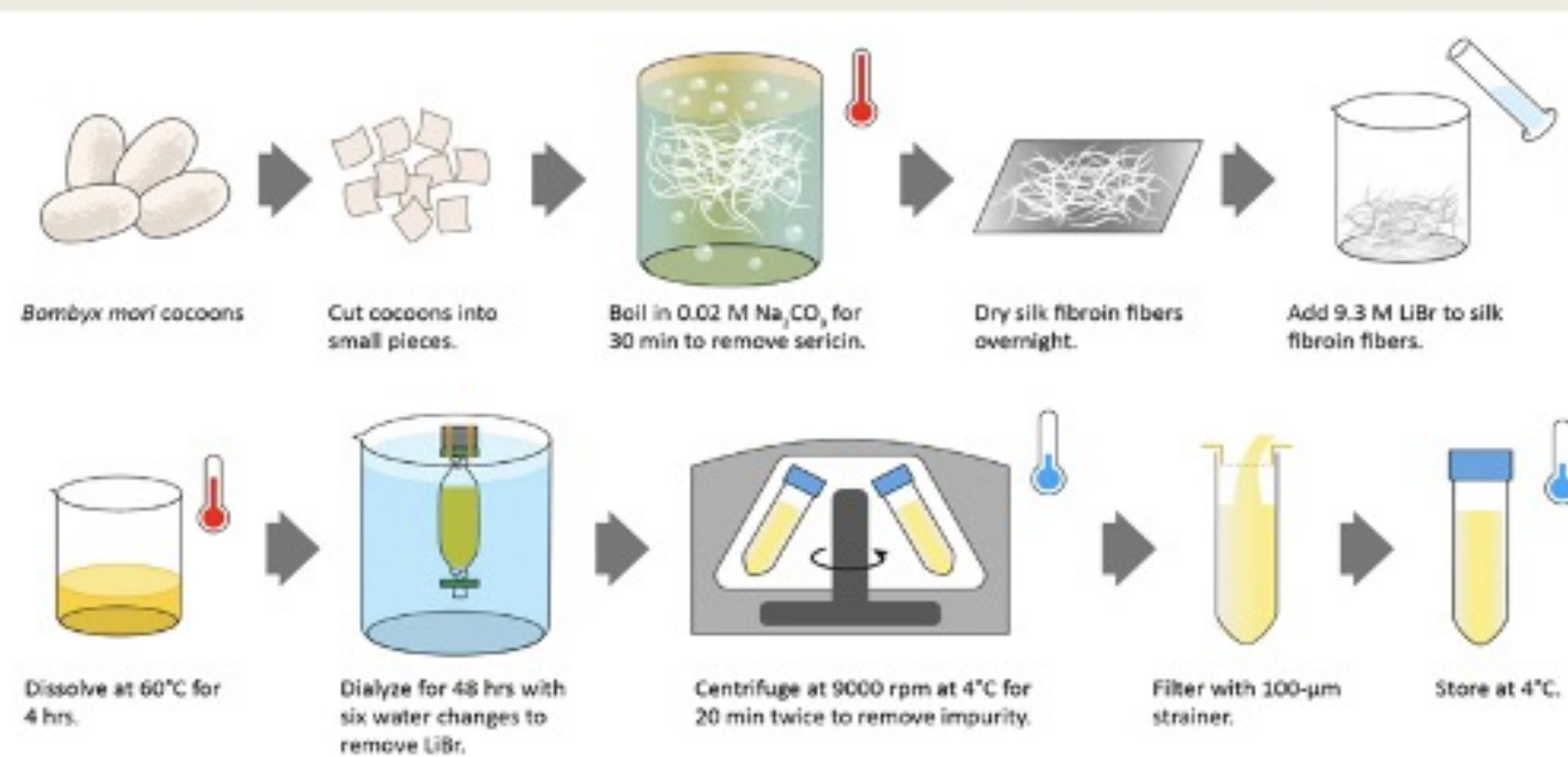


Fig 3. Na₂CO₃ degumming process

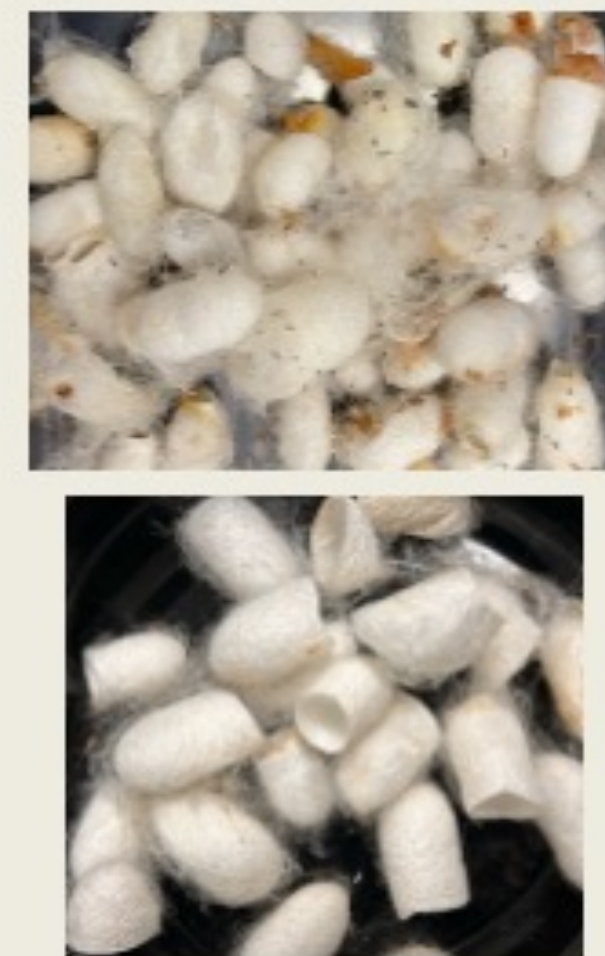


Fig 4. Lab-grown *Bombyx mori* silkworm cocoons (raw material)

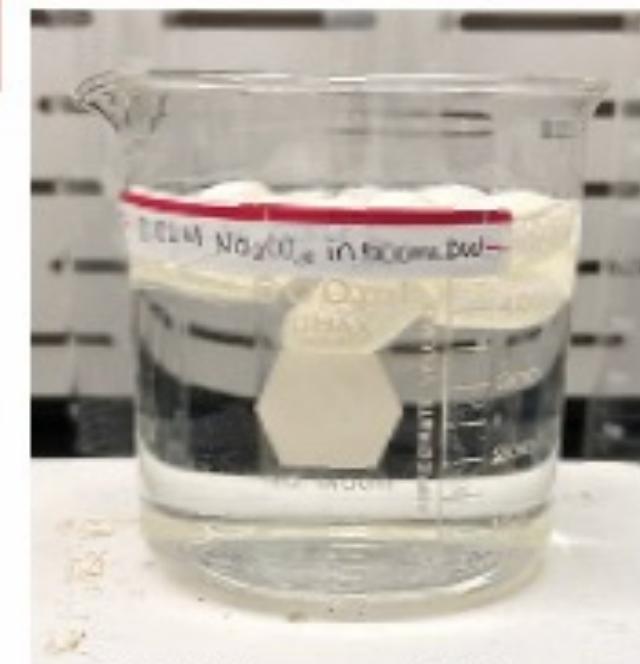


Fig 4. Boiling cocoons in Na₂CO₃



Fig 5. Disintegration of fibers

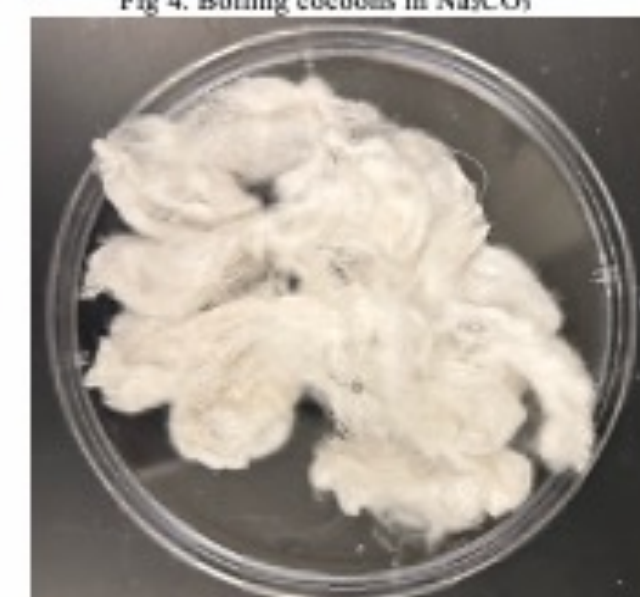


Fig 6. Degummed silk fibers



Fig 7. LiBr dissolution of degummed fibers



Fig 8. Dialysis of silk fibroin



Fig 9. Dialysis of silk sericin

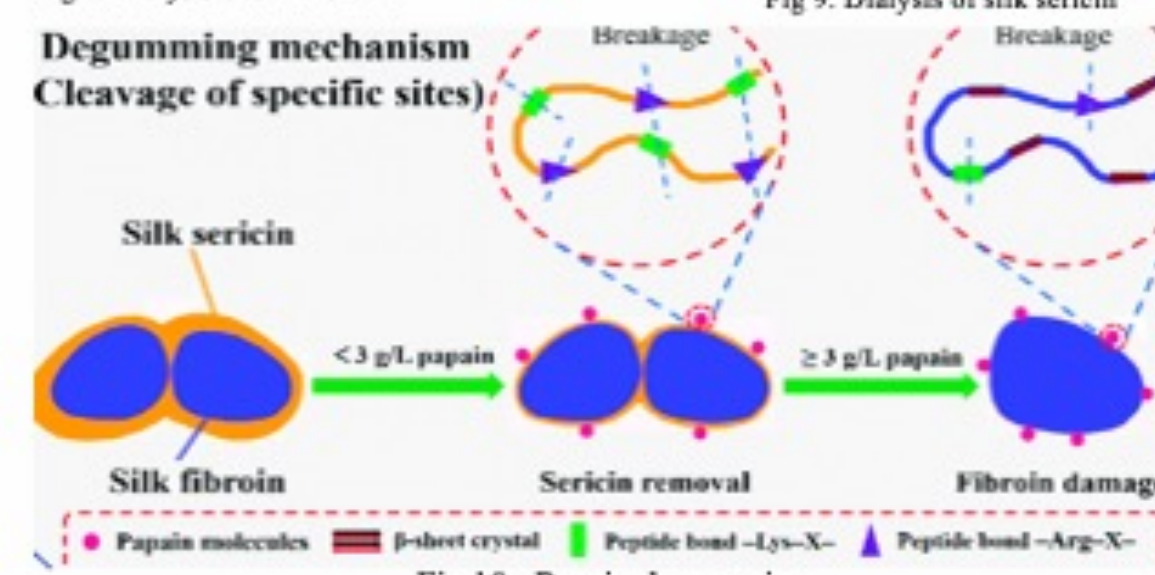


Fig 10. Papain degumming

| | |
|--------------------------------|--------------------|
| Initial mass of cocoons = | 2g |
| Mass of degummed silk fibers = | 1.34g |
| Mass difference = | 0.66g |
| Percent change in mass = | $(0.66) \cdot 100$ |
| | 2 |
| Percent change in mass = | 33% |

Fig 11. Mass difference calculations

Enzyme Kinetics

Michaelis-Menten Enzyme Kinetics

Enzymes are biological catalysts that not only speed reactions but can be used to target the cleavage of specific amino acid bonds of proteins, such as sericin and silk fibroin that can lead to their degradation. This study is focused on two enzymes (papain and protease XIV) that exist in mammalian species including humans. Protease XIV and papain enzymes are going to be used to test the proteins' degradative properties. To determine the concentration of substrate and enzyme, the Michaelis-Menten enzyme kinetics was employed.

- Determines the rate of an enzyme-catalyzed reactions
- Assumes that the concentration of E-S complex is constant
- Specific for each enzyme and substrate
- Allows for calculations of enzyme and substrate concentration

$$v_0 = \frac{(v_{max} [S])}{(K_M + [S])}$$

Fig 12. Michaelis-Menten equation

Sericin's degradative properties will then be determined for its suitability in the fabrication of a scaffold that regenerates the ECM and degrades by the effect of mammalian enzymes.

Sericin Scaffold Suitability for Wound Healing

Cell proliferation will then be assessed using ISO 10993-5 MTT cytotoxicity and proliferation assays which will screen the cell viability response to my tunable sericin scaffold. Research studies conducted on sericin have examined its biocompatibility for its application in wound healing technologies. According to Tsubouchi et al, the addition of sericin to human fibroblast cell cultures resulted in a 250% increased cell proliferation compared to the control group; this indicates sericin's regenerative properties.

Applications of sericin in medicine:

- Wound healing
- Antitumor effects
- Tissue engineering
- Lipid Metabolism
- Drug delivery
- Cryopreservation
- Cosmetology

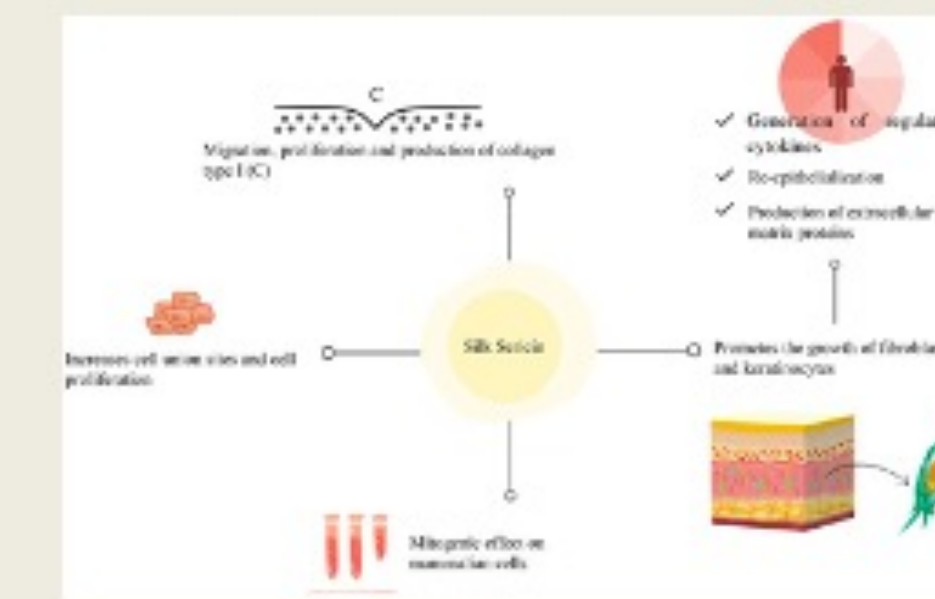


Fig 13. Applications of sericin in medicine

References

Arazavi, Kazemzadeh, S., De-Ekzanke, W., Kani, K., & Sridharan, T. (2019). The Effect of Sericin with Variable Amino-Acid Content from Different Silk Strains on the Production of Collagen and Nitric Oxide. *Journal of Biomaterials Science: Polymer*, 28(9), 1295-1306. <https://doi.org/10.1080/10653162.2019.1645093>

Chirila, Suzuki, S., & McKirby, N. C. (2016). Further development of silk sericin as a biomaterial: a comparative investigation of the procedures for its isolation from *Bombyx mori* silk cocoons. *Progress in Biomaterials*, 5(2), 135-145. <https://doi.org/10.1080/20918001.2016.1191074>

Hartini, & Ibrahim, M. P. (2020). Role of collagen in wound healing. *Drug Invention Today*, 13(1), 55-57.

Tsubouchi, Igarashi, Y., Takawa, Y., & Yamada, H. (2005). Sericin enhances attachment of cultured human skin fibroblasts. *Bioscience, Biotechnology, and Biochemistry*, 69(2), 405-408. <https://doi.org/10.1271/bbb.69.405>

Kiara, Bhanuachari, R. M. C., Ribeiro, L. de F. C., & Nuzari, M. R. M. (2016). Silkwoom Sericin: Properties and Biomedical Applications. *BioMed Research International*, 2016, 8175701-8175710. <https://doi.org/10.1186/s13104-016-0172-0>

ISO 10993-5: Biological Evaluation of Medical Devices - Part 5: Tests for In Vitro Cytotoxicity, 2009