Silk Fibroin Degradation and Sericin Electrospinning for Biopolymer Scaffolds in Bone Regeneration and Orthopedic Implants

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Introduction

Bone fractures have an estimated count of 6.8 million annually, one of the highest categories of fractures in the United States. Surgical hardware used for bone fixation consists of titanium and metal alloy screws, pins, and plates. Despite their clinical utility, metallic surgical implants are associated with an increased risk of infection, tissue toxicity, and unplanned revisional surgeries.

Bombyx mori silk is an emerging naturally-derived biomaterial due to its exceptional mechanical integrity, biocompatibility, and degradative properties of the silk's protein components: silk fibroin (~67 wt %) and sericin (~33 wt%).

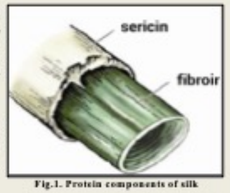


Fig.1. Protein components of silk

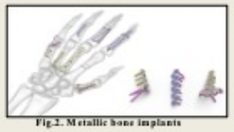


Fig.2. Metallic bone implants

This research effort is aligned with a joint project between Mayo Clinic's Dr. Erwin A. Kruger and the BioICAS lab, focused on the development of rationally-designed degradable and tissue regenerative, bioactive polymer-ceramic nanocomposite surgical hardware for regenerative surgery applications.

Research Aims:

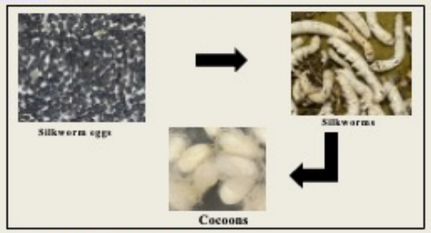
1. Isolation of sericin and silk fibroin polymers from the coaxial arrangement of *Bombyx mori* silkworm cocoon fibers.
2. Enzymatic degradation study of silk fibroin films using Protease XIV.
3. Production of 3D nano-scaffolds using naturally-derived *Bombyx mori* sericin polymer.
4. Evaluation of biocompatibility assessment of electro-spun sericin scaffolds.

Fig.3. Medical applications of silk

Silk Fibroin

Step 1: *Bombyx mori* silkworm farming

Method: Lab-grown, domestic setup



Step 2: Isolation

Method: Alkaline Degumming


Fig.4. Alkaline Degumming of *Bombyx mori* silkworm cocoons

Step 3: Silk Fibroin Films

Method: Film casting followed by dehydration

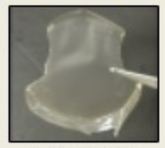


Fig.5. Silk Fibroin films

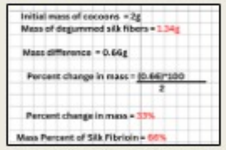


Fig6. Mass difference of degummed fibers, maximum sericin loss as measured by weight reduction.

Step 4: Characterization

Method: Fourier Transform Infrared Spectroscopy (FTIR) & Optical Microscopy

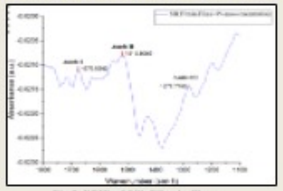
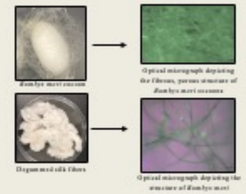


Fig.7. FTIR of Silk Fibroin films



Step 5: Enzyme Degradation Study

Method: Michaelis Menten Enzyme Kinetics

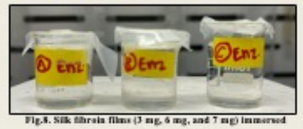


Fig.8. Silk fibroin films (3 mg, 6 mg, and 7 mg) immersed in Protease XIV enzyme for 48 hours

$$v = \frac{V_{max}[S]}{K_M + [S]}$$

Michaelis-Menten Enzyme Kinetics Equation Used for calculating Protease XIV enzyme concentration (E0) for SF degradation tests

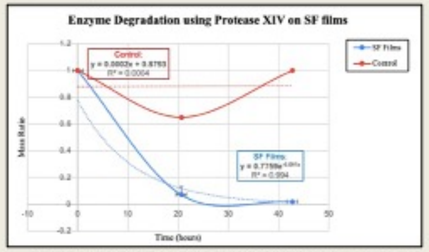
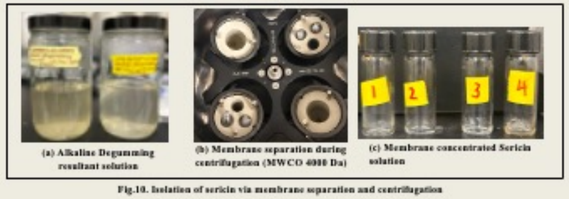


Fig.9. Mass loss of silk fibroin films over a 48-hour period, with the blue curve representing films in proteolytic, non-enzymatic Protease XIV enzyme and the red curve as the control. A regression line plotted demonstrates the trend of the curves.

Sericin

Step 1: Isolation of sericin

Method: Membrane separation followed by centrifugation



(a) Alkaline Degumming resultant solution
(b) Membrane separation during centrifugation (MWCO 4000 Da)
(c) Membrane concentrated Sericin solution
Fig.10. Isolation of sericin via membrane separation and centrifugation

Step 2: Sericin Films

Method: Film casting


Fig.11. Sericin film made by film casting

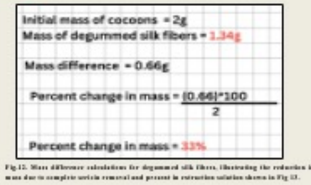


Fig.12. Mass difference calculations for degummed silk fibers, illustrating the reduction in mass due to complete sericin removal and present in extraction solution shown in Fig.11.

Step 3: Characterization

Method: Fourier Transform Infrared Spectroscopy (FTIR)

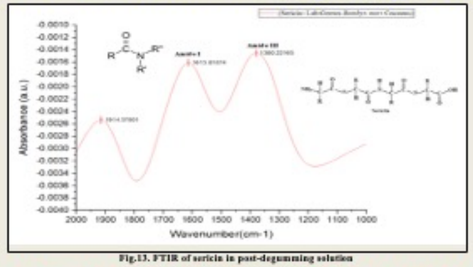


Fig.13. FTIR of sericin in post-degumming solution

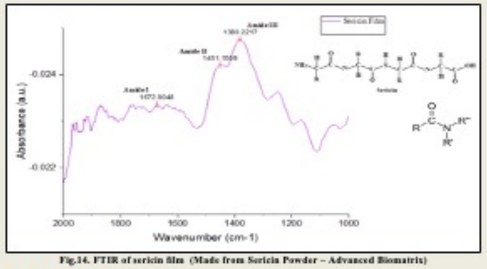


Fig.14. FTIR of sericin film (Made from Sericin Powder – Advanced Biomatrix)

Step 4: Scaffold Formation of Sericin Solution

Method: Electrospinning

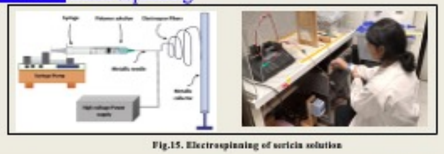


Fig.15. Electrospinning of sericin solution

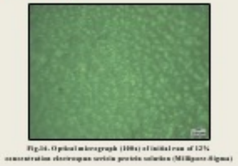


Fig.16. Optical micrograph (100x) of initial run of 12% concentration electrospun sericin protein solution (Millipore Sigma)

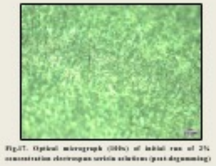


Fig.17. Optical micrograph (100x) of initial run of 3% concentration electrospun sericin solution (post degumming)

Ongoing and Future Work

1. Determination of optimal process parameters for electrospinning of sericin
2. Biocompatibility testing using ISO Standard 10993#5 Tests for In Vitro Cytotoxicity of NIH 3T3 cells seeded on sericin electrospun scaffolds
3. Enzyme Degradation study of Sericin Films with mammalian enzyme: α-chymotrypsin

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