Assessing Natural Silk's Suitability for Bioengineering Next Generation Fracture Fixation Devices

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Introduction

The potential use of silk fibroin (SF) as a biomaterial for use in bioengineering fracture fixation devices has garnered significant attention in recent years, mainly due to SF's robust mechanical properties, excellent biocompatibility, and potentially controllable degradation rate. The specific aims of this project include:

1. Domestically farm *Bombyx mori* silkworms from egg to cocoon stage.
2. Develop silk fibroin extraction process (degumming) and degradation test system for assessment of chemical and enzymatic degradation methods
3. Determine silk fibroin chemical/enzymatic degradation profiles

Methods

Domestic Farming of *Bombyx mori* Silkworms

Silkworms undergo four stages of development (~30- 40 days)

- **Egg Hatching**: Maintaining temperature (21°C - 28°C) & humidity (80% -90%); hatched 250 silkworm eggs (hatching period: ~ 7-8 days)
- **Larval Development**: Post-hatching, requires a constant supply of artificial silkworm chow (mulberry leaves, water, vitamins, etc.) or mulberry leaves; undergo four stages of development (Fig. 2- Fig. 7)
- **Larval Rearing**: Temperature, humidity, nutrition, and cleanliness for 21-28 days were maintained during growth of larvae.
- **Cocoon Production**: Cocoon production occurs after the fourth stage of larvae development where the larvae weave the silk threads around their body, forming cocoons (Fig. 1).


Fig 1. Cocooning stages of silkworms

Silk Fibroin Degumming Extraction Technique

Silk fibroin is composed of two protein structures: sericin and silk fibroin; a process called degumming is employed to isolate the silk fibroin from the sericin coating. Techniques used for degumming include:

Alkaline Degumming (Fig. 10):

- 0.1-0.5% Na_2CO_3 solution at 100°C for approximately 30-60 minutes. Effectively isolates sericin but degrades the structural integrity of the silk fibroin.
- Widely used for commercial processing of *Bombyx mori* cocoons.


Fig 2. Egg stage of *Bombyx mori* silkworms

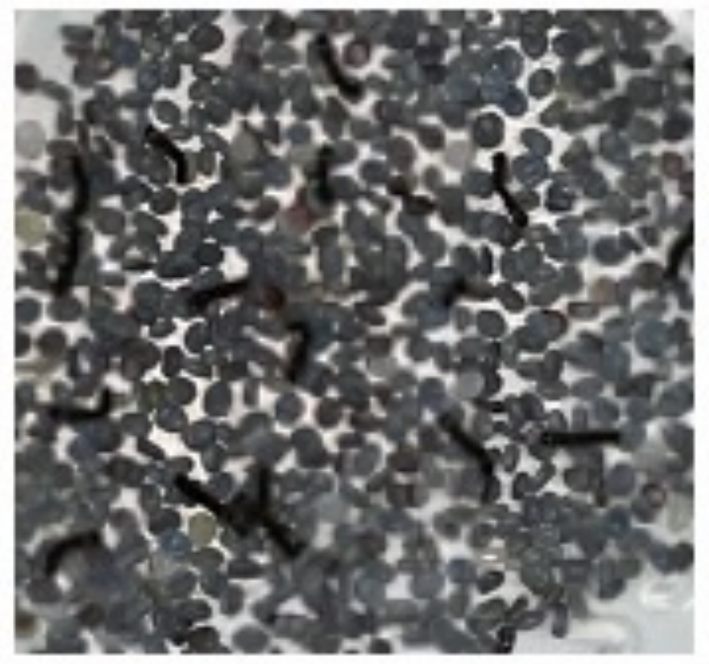
Fig 3. Newly hatched silkworm eggs


Fig 4. First instar stage of silkworms


Fig 5. Second instar stage of silkworms


Fig 6. Third instar stage of silkworms


Fig 7. Fourth instar stage of silkworms


Fig 8. Setup for cocooning


Fig 9. Precursor silk threads formed by fourth instar silkworms

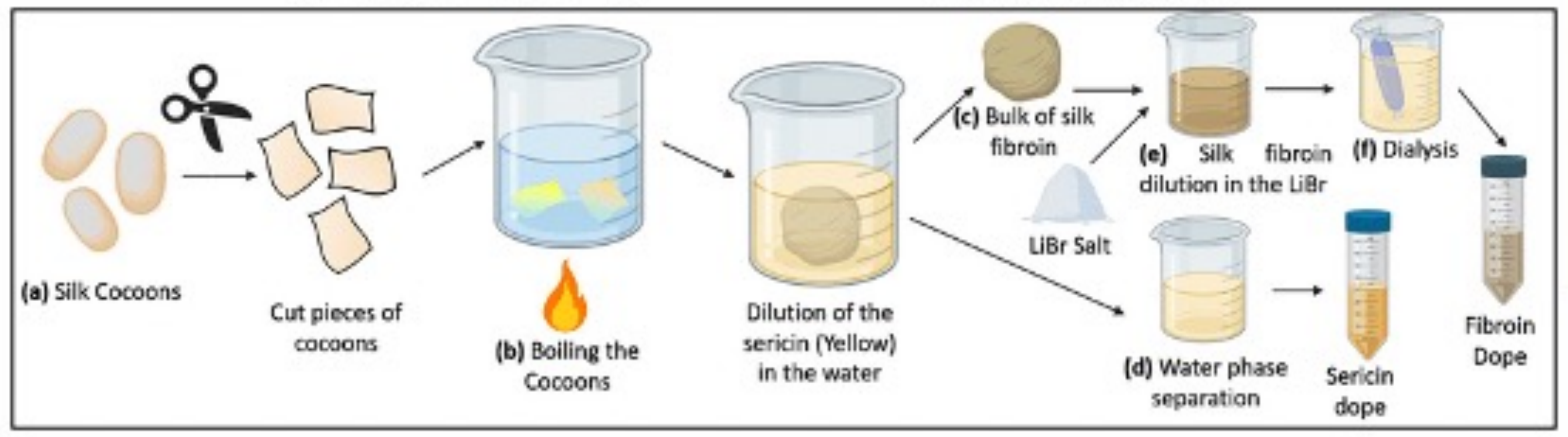

Fig. 10 Degumming process to extract silk fibroin from silk threads.

Degradation Studies

Investigating the degradation of SF is essential to determine its durability over time and its application in medical devices. Three types:

1. **Chemical Degradation:** Fenton's Regent & Hydrogen Peroxide (H_2O_2)
2. **Proteolytic enzymes (e.g. papain & proteases):**
   - Degrades specific parts of the sericin chain as the proteolytic enzyme papain works to cleave specific amino acids of the SF chain.

Results

Silkworm Farming

Egg Hatching Larval Development: Eggs transitioned from a dark grey color to a pale blue, indicating the development of the eggs to larvae.

Larval Development (Fig. 2 - Fig. 7)**:** Hatched as black-colored larvae (~1 inch long); underwent moulting to develop into paler, larger larvae (~3-4 inches).

Cocoon Production (~3-8 days)**:** Early in the fourth instar stage, silkworms ejected a pre-cursor liquid (Fig. 9) to clean silk glands in preparation for silk production. Setup as displayed in Fig. 8

Silk Fibroin Extraction

While awaiting to harvest the cocoons, both the silk fibroin extraction method (Fig. 10) and the enzymatic degradation processes have been established.

Discussion

Now, on day 31 of the development process, the larvae are in their 4th instar stage, having already formed precursor silkworm threads (Fig. 9) and poised to spin cocoons.

As the cocoons take about 3-7 days to form, the research will address aims 2 and aim 3 next.

References