Silk-based Drug Carriers for Pulmonary Drug Delivery

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Background

- **Silk proteins** are recently attracting immense attention in biomedical applications, due to their natural origin, excellent biocompatibility, ease of processing, outstanding mechanical properties and biodegradability[1,2].
- **Fibroin**, the major protein in silk, has already been widely established as a platform for controlled drug delivery, tissue regenerations, as well as for applications to cancer therapy[1,3,4].
- Nevertheless, silk biomaterials have not been formulated for pulmonary drug delivery.
- **The aim of this project** is to develop novel, biologically-inspired silk-based inhalable drug delivery system that targets lung diseases.

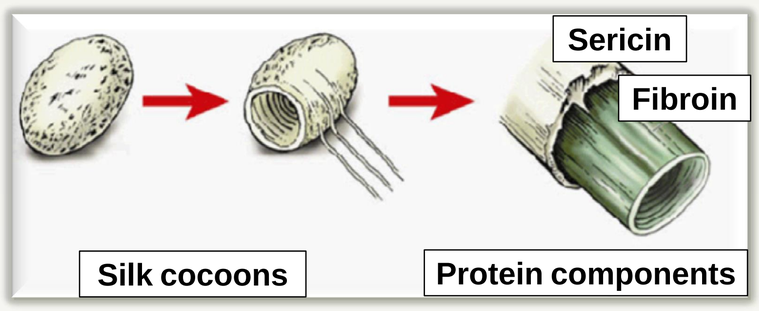

Figure 1. Protein components of silk cocoons

Methods

Formulation
- **Silk proteins** from cocoons of *Bombyx mori* (Bm) & *Antheraea mylitta* (Am) silkworms (Fig. 2)
- Spray-drying (SD)
- Spray-freeze-drying (SFD)

Characterisation
- Morphology – Scanning Electron Microscopy (SEM)
- Topography – Atomic Force Microscopy (AFM)
- Size analysis by laser diffraction
- Crystallography – X-ray Diffraction (XRD)

***In vitro* studies**
- Aerosolisation performance (Fig. 3)
- Quantification of particles
- Cell culture & biocompatibility (A549 lung cell line)

Figure 2. Silk worm and silk cocoon[5]

Figure 3. Spray-dried Bm fibroin dispersed using Next Generation Impactor (NGI)

Results & Discussion

Particle Characterisation:

Both spray dried and spray-freeze-dried silk particles portrayed the potential for drug encapsulation and use as drug carriers (Fig. 4). Surfaces of particles were rough and porous, beneficial for successful lung deposition.

Laser diffraction for particle size analysis for spray-dried Am fibroin and Bm fibroin showed that 50% of the particles had geometric diameters less than 7.03 µm and 5.20 µm, respectively.

XRD results showed that all particles were amorphous, regardless of method of production.

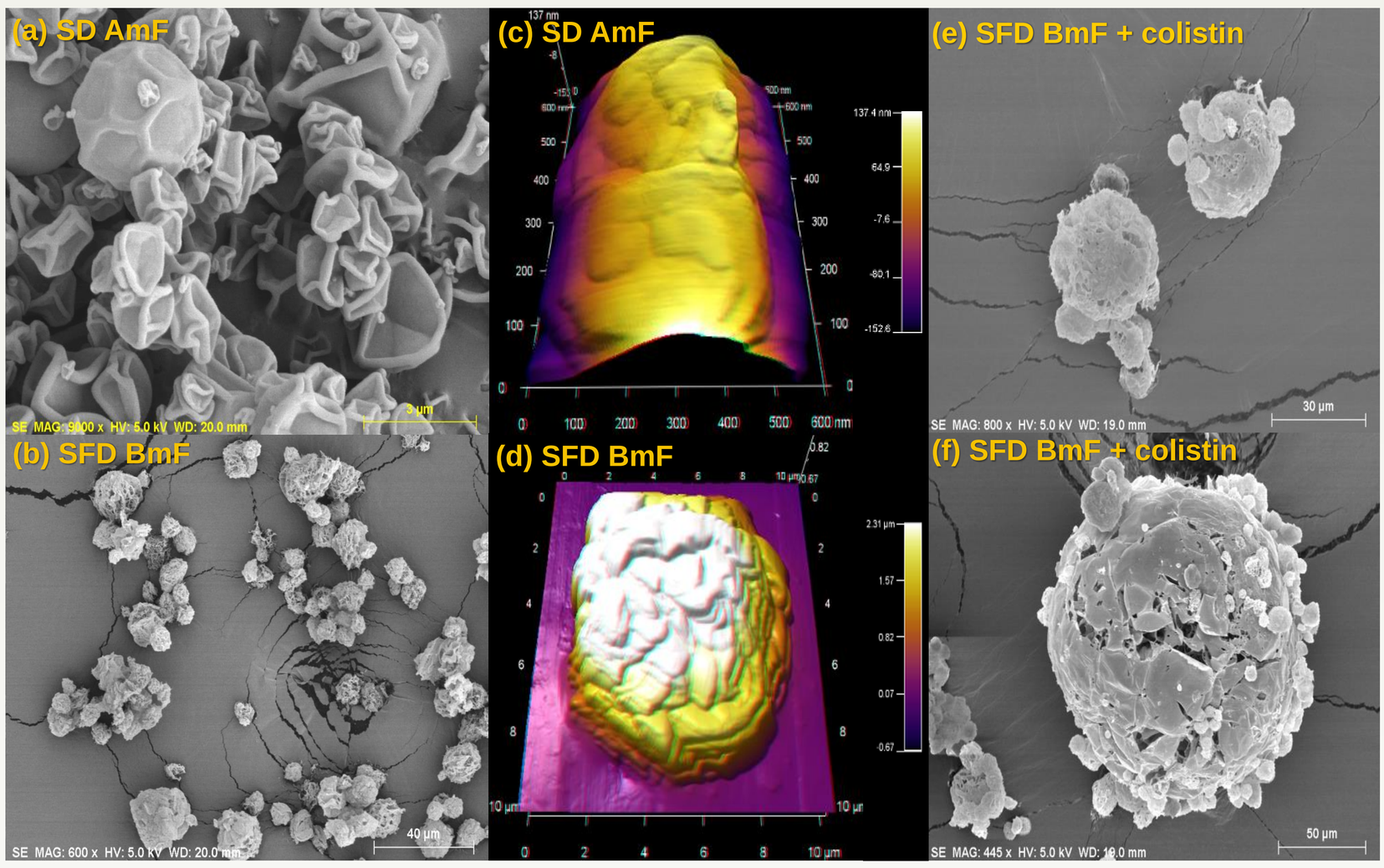

Figure 4. SEM & AFM images of particles: (a)(c) spray-dried Am fibroin 2% (b)(d) spray-freeze-dried Bm fibroin 0.5% with trehalose 0.5% (e)(f) spray-freeze dried Bm fibroin with colistin at concentration ratio of 10:1

Aerosolisation Performance:

The dispersible, inhalable powder were compared for each method of productions (Fig. 5). The difference in fine particle fraction (FPF) for spray dried and spray-freeze-dried Bm fibroin particles at 60L/min for 4 sec were minimal - 61.67% and 62.25% respectively.

However, the production yield of powder varied, 48.69% and 61.26% respectively, therefore spray-freeze-drying is the method of choice for Bm fibroin powder for inhalation.

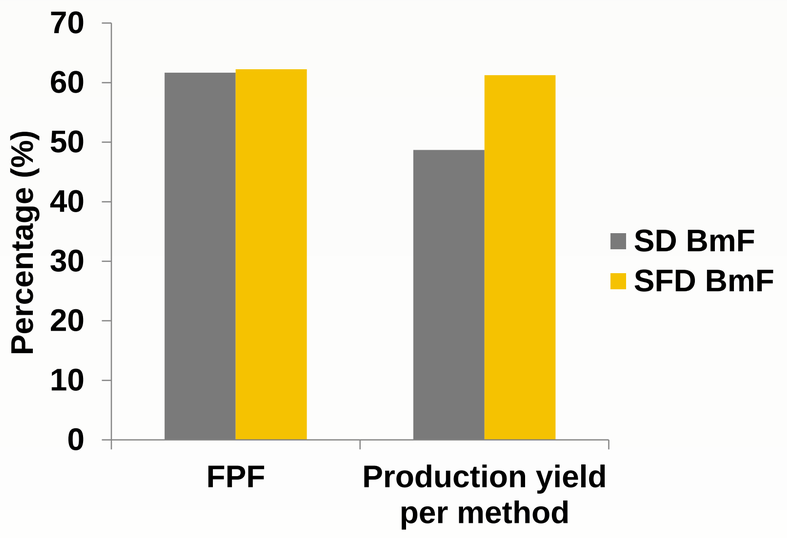

Figure 5. Comparison of fine particle fraction (FPF) and yield of production from spray-freeze-drying (SFD) & spray drying (SD)

Cell Culture:

Behaviours of cell attachment to the 2D silk films were comparable to tissue culture plate (TCP) and demonstrated that both Am and Bm fibroin materials were not cytotoxic to the A549 lung cells (Fig. 6).

This is consistent with the results of our preliminary studies of biocompatibility using alamarBlue and MTT assays.

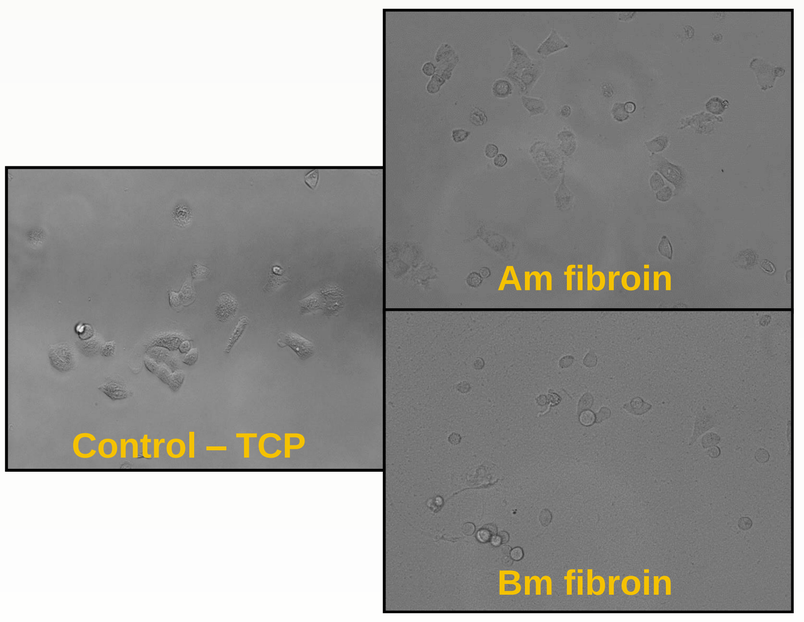

Figure 6. A549 cell attachment at 5 hours post-seeding to 2D silk fibroin films

Expected outcomes

- New silk-based nanomatrix for pulmonary delivery system
- Application to specific lung disease model
- Silk proteins assist regeneration of tissues during recovery from lung diseases

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5. Picture: Silkworm_cocoon blogs.discovermagazine.com