Spider Silk Biotechnology

Spider silks are protein-based "biopolymer" filaments secreted by specialized epithelial cells of the spigots of a spider's spinneret (Fig. 1). Spider silk has not been used commercially because of the difficulty of farming spiders. Nexia Biotechnologies (Quebec, Canda; www.nexiabiotech.com) has developed a proprietary silk production system which has proven to be successful in producing the most authentic, man-made spider silk to date. The result is "BioSteel®", a family of recombinant spider silk proteins. Nexia uses a type of spider silk called the dragline. Dragline silk is the fiber from which spiders use to make the scaffolding of their webs. It has been estimated by scientists to be at least five times as strong as steel, twice as elastic as nylon, waterproof and stretchable. Dragline spider silk is actually stronger than Kevlar synthetic fiber. The dragline spider silk fibers offer unique combinations of properties suited to a range of specific technical applications. The industrial markets for the silk include protective clothing (e.g., ballistic protection), speciality ropes and nets, and bio-compatible sutures for the medical industry.

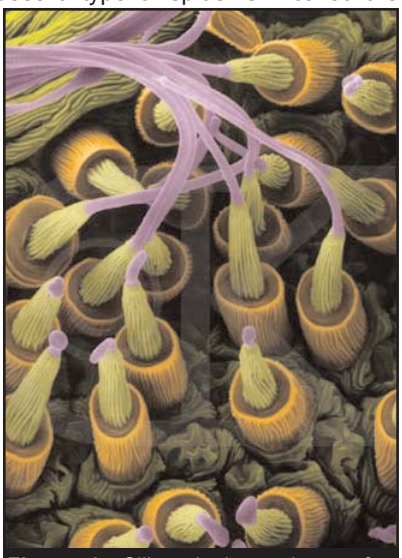
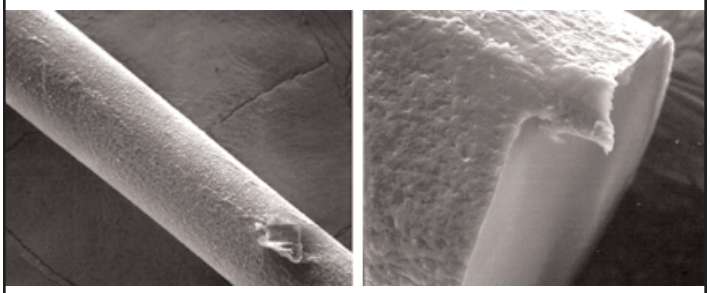


Figure 1: Silk spinning spigots of a spider spinneret. The purple threads are the silk fibers.

Nexia is mimicking the process of spider silk production by expressing the the dragline genes (*ADF-3/4* and *MaSpI/II*) in mammalian cells (Ref. 1); These genes were isolated from the two spider species *Araneus diadematus* and *Nephila clavipes* (Fig. 3), respectively. Through this process they were able to produce dragline silk proteins with molecular masses of 60 to 140 kilodaltons. The protein core of dragline silk fibers are secreted as a mixture of two soluble proteins from the epithelial of the major ampullate glands of the spider. The dragline silk genes encode proteins that form crystalline alanine-rich stretches (ASAAAA) responsible for the silk's mechanical function, which are embedded with glycine-rich amorphous regions (GGYGPG) implicated in providing the elasticity in the silk filament. Immortalized bovine mammary epithelial alveolar cells (MAC-T) were chosen as expression systems for the *ADF-3/4* and *MaSpI/II* genes, because they excel at secreting proteins. Figure 2 shows scanning electron microscopy (SEM) images of the recombinant silk protein produced from the ADF-3 transfected MAC-T cells.

Figure 2: Recombinant Silk Protein.



X500 X2000

Scanning Electron Microscopy Images

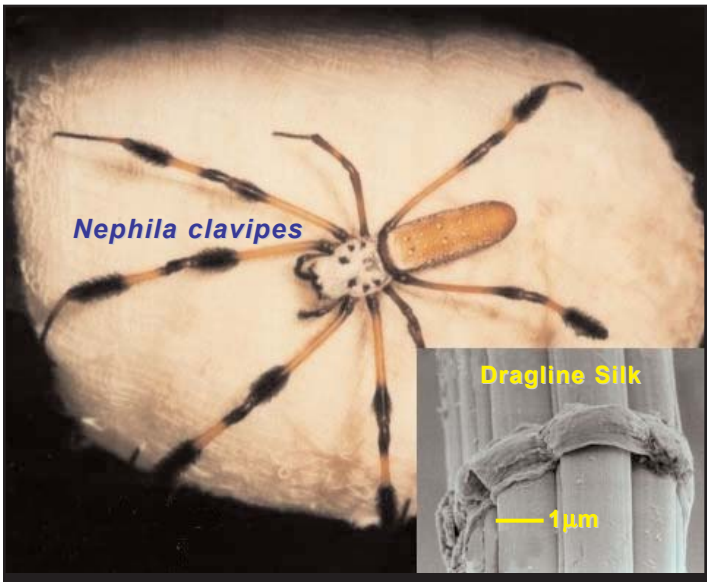


Figure 3: The dragline silk of the *Nephila clavipes* spider.

The recombinant ADF-3 proteins were first concentrated in water, then it were drawn through methanol to precipitate and form continuous fibers. The silk spun from the *ADF-3* gene alone produced a dragline silk with a molecular mass of 60kD. Although the ADF-3 recombinant silk was very strong, some mechanical properties, such as elasticity, were not quite good as that of native spider dragline silk. The difference between the synthetic silk and native silk may be due to the fact that the native silk is composed of two proteins, ADF-3 and ADF-4 or MaSpI and MaSpII depending on the species, whereas the synthetic silk was only composed of ADF-3. The success of this cell-based prototype system has encouraged Nexia to continue the scale-up of manufacturing spider silk protein within its transgenic BELE® (Breed Early, Lactate Early) goat system. Dairy animals have utility in this application because they have a very large number of mammary cells, which are ideal for producing the silk. The small physical size of BELE goats reduces feed and housing costs. The biggest expense of a ruminant transgenic program is the number of recipient animals used. The BELE goat model accelerates the breeding time and genetic progress and reduces the total cost of the transgenic program. The BELE goats produce approximately 1L of milk/d for the 305-d lactation period which is sufficient for production of kg amounts of recombinant proteins, useful for prototype manufacturing or clinical studies.

Recombinant spider silk proteins have also been produced in bacteria and yeast systems with limited success (Ref. 2). Another method to produce these silk proteins is through their expression in transgenic plants (Ref. 3). The recombinant silk proteins produced form bacteria, yeast, and plant often yielded insoluble proteins, which clumped within the cell, and which did not have the tenacity of the silks produced in mammalian cells.

References:

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