Biomimetic spider silk fibres: from vision to reality

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Silk is a well-known and unique material that has been used in different applications for more than 5,000 years. The shiny silk fabrics that we know are actually made of threads created by the Chinese silkworm, but various arthropods can produce silks. Spider silk, for instance, is known to be the toughest fibre on Earth, outperforming both naturally and human-made fibres. Due to the problems of farming typically cannibalistic spiders, biotechnological approaches have been developed for producing spider silk proteins, which can be subsequently spun into biomimetic fibres. Here, we discuss recent developments in recombinant spider silk production and processing.

Silks exhibit various functionalities that depend on the type of natural application, e.g., as the frame of a web, protection of offspring and wrapping of prey. Insects are able to produce silks in different glands such as Malpighian tubules, labial or dermal glands with different structures and functions (coiled-coil, extended β-sheet, cross-β-sheet, collagen triple helix, polyglycine II). In the case of orb-weaving spiders,
up to six different types of silks can be obtained, all originating from separate glands. Figure 1 represents the different silk types and their functions in such spiders. As shown, a seventh gland produces a silk-like glue (aka aggregate silk), which is deposited on the web to retain prey within it. The most analyzed type of silk is the major ampullate silk, aka dragline silk, used for creating the frame of webs and as a lifeline. Dragline silk fibres have outstanding mechanical properties such as tensile strength, comparable on a weight to weight basis with that of steel, in conjunction with high elasticity. The combination of these two properties renders silk a material with a toughness that is at least two-to-three times higher than that of synthetic fibres such as Nylon or Kevlar. The silk's features can be controlled by their amino acid sequences (rich in alanine, serine and glycine), but also via the processing of the silk dope. The arrangement of spider silk proteins, aka spidroins, during fibre assembly are the basis of the extraordinary mechanical properties of the fibre. The non-repetitive terminal domains exhibit an α-helical secondary structure and have an important function during the initiation of fibre formation.

**Hurdles to mimicking spider silk fibres**

There are several difficulties associated with using natural spider silk. First is its availability. Large-scale farming of spiders is not possible, as most species are territorial and cannibalistic. Further, harvesting silk directly from captured spiders typically yields fibres with lower properties than that of free-living ones. Moreover, batch-to-batch variation and impurities hamper their applicability, which initiated research on the recombinant production of spidroins. Nowadays, the biotechnological production of spidroins is well established.

**Recombinant spider silk technologies**

Recombinant spider silk production requires several steps including: DNA sequence determination, DNA design or engineering for selected production hosts and, upon gene expression, the purification of the recombinant proteins. Different organisms such as bacteria, tobacco plants, yeast, silkworms, goats, insects and mammalian cells have been used to
produce recombinant spidroins. However, the most frequently used host organism is *Escherichia coli*, which enables fast and high-density cultivation, as well as the inexpensive and large-scale production of spidroins. Therefore, in our research group we use *E. coli* to recombinantly produce spidroins – for example, based on two different *Araneus diadematus* MaSp2 proteins. Another approach is using transgenic animals as hosts to produce recombinant spidroins in secreted body fluids. For example, the Lewis research group at the University of Wyoming has developed a way to incorporate a spider’s silk-producing genes into goats, which secrete the spidroins into the milk. The spidroins have been used for medical applications such as suture threads and scaffolds for tendon regeneration as well as to fabricate bulletproof wear.

**Biomimetic fibre processing and putative applications**

Mimicking the structure of spider silk fibres and fabricating fibres with nature-like mechanical properties has been another focus of much research. The fibre’s properties are not only based on the underlying proteins (spidroins), but also on their processing, including biomimetic self-assembly within the silk dope followed by a highly controlled spinning process. In the spinning dope, spidroins assemble into micelles, and the dope exhibits liquid crystal-like properties. The arrangement of spidroins in micelles is an extremely stable storage form suppressing unwanted fibre formation. As soon as the spider needs a fibre, it can press the dope into the S-shaped tapered spinning duct. Here, a process akin to salting out will remove water, and at the same time the pH value is reduced (from approximately 7.2 to 5.7). Finally, shear forces resulting from the tapering of the spinning duct and pulling forces will orient the spidroins within the resulting fibre (Figure 2).

Due to the complex process of natural spinning and pre-assembly of spidroins, the technical production of spider silk fibres has been challenging. Techniques to fabricate spider silk fibres are biomimetic or wet-spinning approaches. Therein, a spinning dope is extruded into a coagulation bath and fibres will be formed and collected from the bath. In wet-spinning, spider silk fibres can be spun from both aqueous and organic solvents, but the latter does not allow the formation of the delicate hierarchical structure needed to obtain the extraordinary toughness of natural spider silk. So far, only biomimetic approaches (out of aqueous solutions) have yielded fibres with a toughness equaling that of spiders, and the preparation of a self-assembled spinning dope is crucial. The toughness of these fibres, made of a MaSp2 derivate based on the sequences of *A. diadematus*, were identical to that of natural *A. diadematus* dragline fibres.

Finally, there are also commercial approaches to yield technically produced spider silk-like fibres (see box).

In summary, the recombinant production of spidroins, and processing into fibres that exhibit properties similar to those found in nature, is possible. This could be the beginning of an era of exploring the potential applications of these fascinating materials.
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