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Biotechnological Uses of Spider Silk

by Omar Bashqawi

(Biology 1130)

Spider silk is an amazing biological construct that has incredible mechanical properties which are ideal for the pursuit of biomaterials. It is biocompatible, biodegradable, has high tensile strength, and great elasticity. Since natural spider silk is difficult to obtain in large quantities, recombinant spider silk protein is used for testing and experimentation. Biotechnological applications being experimented with currently include: coatings, films, hydrogels, particles, non-woven meshes, drug carriers, and medical sutures. Future uses of perfected recombinant spider silk proteins could also include biomaterials such as superbly tough clothing, armor, and construction materials.

According to Eisholdt et al. (2011), there are seven different types of spider silk that can be created, depending on species and environment: Major Ampullate Silk, Flagelliform Silk, Aggregate Silk, Minor Ampullate Silk, Pyriform Silk, Aciniform Silk, and Cylindriform Silk (Figure 1). These different forms of silk expressed by spiders play different roles based on function, environment, and spider. All spider silk proteins are composed of one or more proteins called Spidroins, which share a primary structure pattern comprised of a large central core of repeated modular units. Approximately 90% of the amino acids are used to compose these modular units (Eisholdt, Scheibel & Smith, 2011).

Between the different silk types their strength (the stress needed to break the fiber) ranges from 0.02 to a remarkable 1.7 GPa, which exceeds steel (1.5 GPa), while the extensibility varies between 10 and 500%. Interestingly, most spider silks have a sophisticated combination of strength and extensibility, which yields a very high toughness (the amount of energy absorbed per volume before breakage) that exceeds most natural or man-made fibers. Also common for all silks is their viscoelastic behavior, since upon stretching energy is dissipated in the form of heat, diminishing any elastic recoil (Eisholdt et al., 2011).

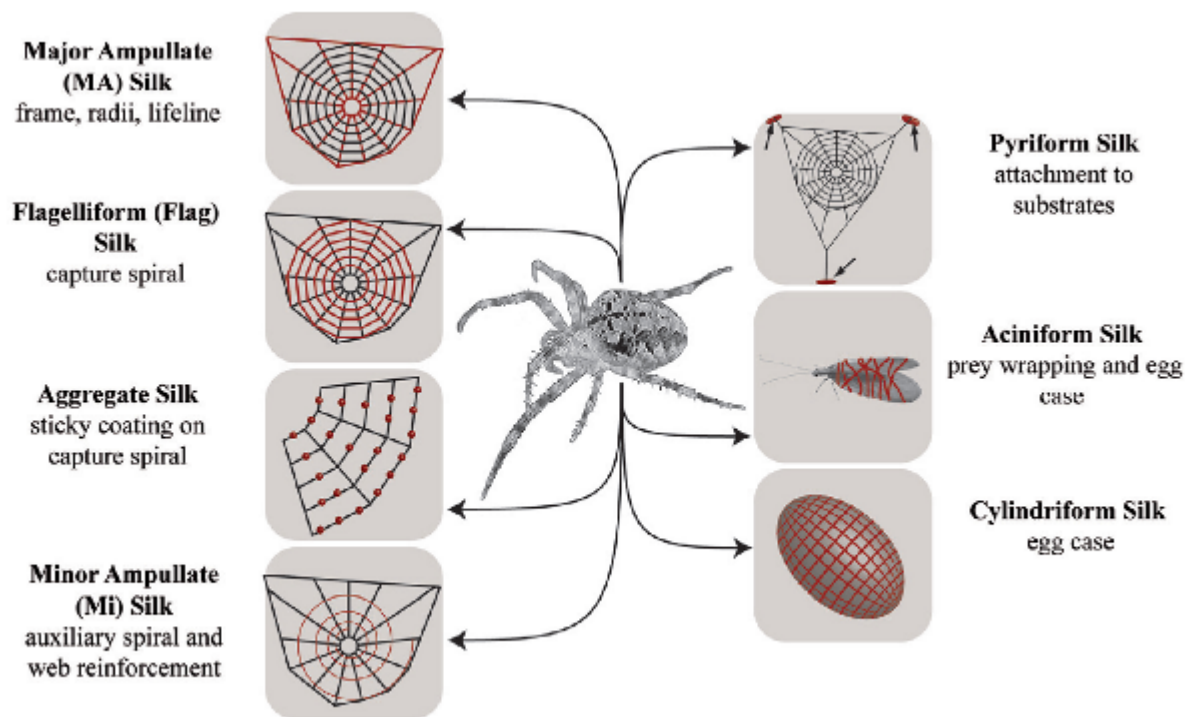


Fig. 1 Schematic overview of different silk types produced by female orb weaving spiders (*Araneae*). Each silk type (highlighted in red) is tailored for a specific purpose. (Eisholdt et al., 2011)

Due to the difficulty in obtaining natural spider silk, from issues such as time consuming collection of webs and difficult farming techniques, another method of gathering silk is needed. Recombinant Spider Silk Protein (RSSP) is the answer to produce nearly identical, natural spider silk protein. For example, Eisholdt et al. describe that the expression of these silk proteins through recombinant processes (mainly through *Escherichia Coli*) allow for the creation of protein structures that are not natural. Examples include: spheres, capsules, films, non-wovens or hydrogels (Figure 2). These non-natural structures have great potential for various applications (Eisholdt et al., 2011).

There are many methods in use to produce these recombinant proteins, each with varying physiological traits and homogenous properties. Tokareva et al. (2013) details that RSSP has been created through a variety of methods including: bacteria (*Escherichia Coli*), yeast (*Pichia pastoris*),

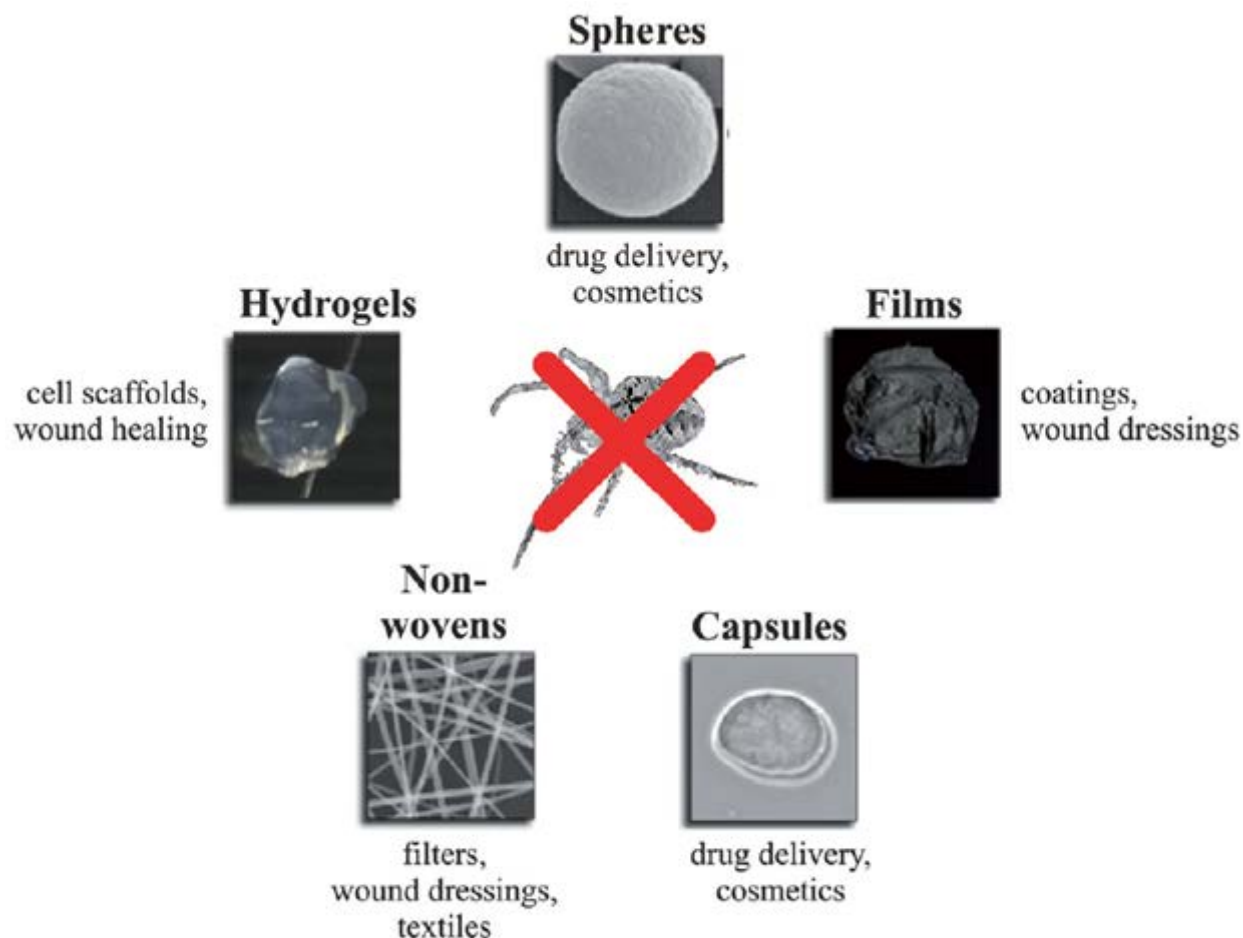


Fig. 2

(Eisholdt et al., 2011)

plants (soybean, tobacco, *Aribidopsis*), insects (silkworm larvae), mammalian cell lines (BHT/Hampster), and transgenic animals (mice, goats). The process to create the recombinant proteins followed four steps: first, design and assemble synthetic silk-like genes into genetic 'cassettes'; second, insertion of the segment into a DNA vector; third, transformation of the recombinant DNA molecule into a host cell; fourth, expression and purification of the clones (Figure 3) (Kaplan, Michalczechen-Lacerda, Rech & Tokareva, 2013).

The different RSSP proteins created through different production methods varied in molecular composition, form, length, tensile strength, and possible applications. A difficulty represented throughout most of the referenced material in this paper was the fact that RSSP created through bacterial methods yielded the greatest amount of protein but the lowest availability of stranded proteins. Use of *Escherichia Coli* to create RSSP is the quickest easiest method to create a sizable supply of RSSP. However, this bacterium suffers from the ability to create long strands of protein. The use of yeast and mammalian cell lines produced longer, more complex protein strands of RSSP but at lower quantities (Tokareva et al., 2013).

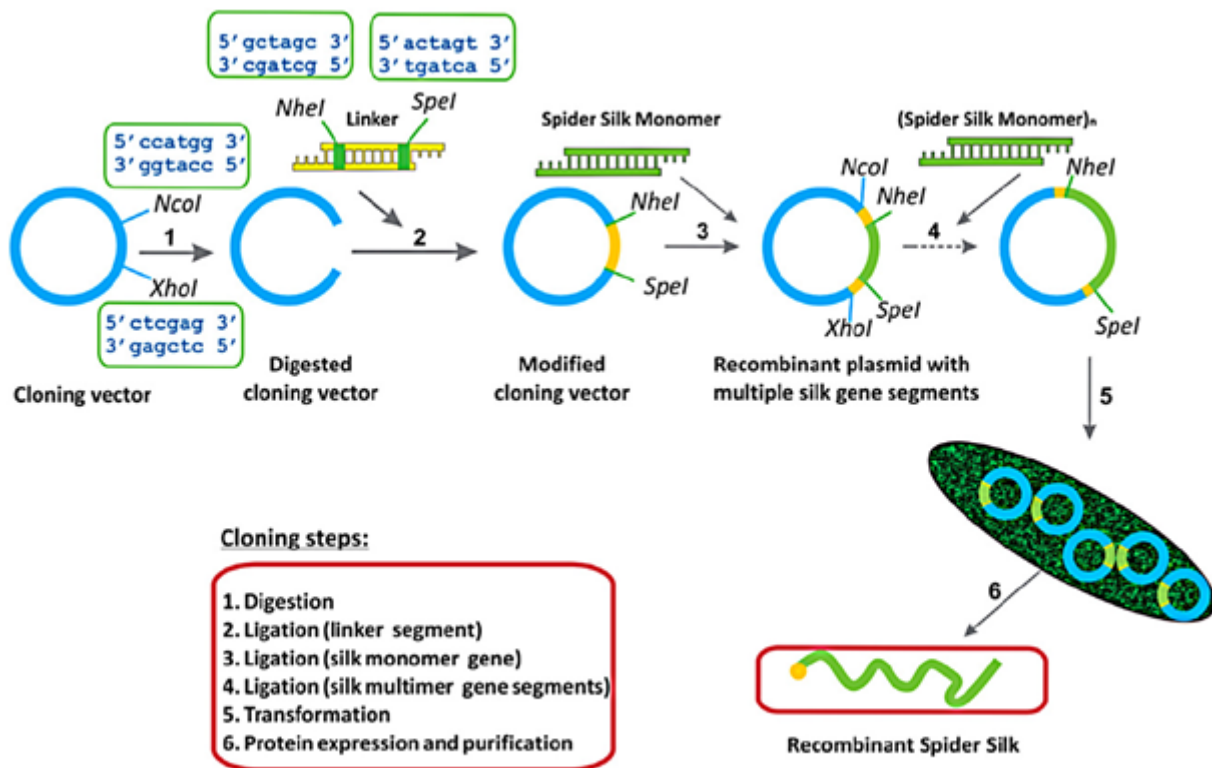


Fig. 3 Recombinant DNA approach used to prepare silk-like proteins.

(Tokareva et al., 2013)

According to Kuwana et al. (2014), use of transgenic silkworms to produce spider silk proteins yielded greater quantities of RSSP in nearly identical composition to that of natural spider silk. They incorporated spider silk protein genetic information into p-HC enhanced green fluorescent protein (EGFP) plasmids, along with other test samples (Figure 4). After propagation of the transformed silkworms, results showed evidence that the transgenic spider silk protein was nearly equal in tensile strength and application. They were able to weave fabric composed of the recombinant silk with greater strength and toughness than that of standard silkworm silk. Implications show that it is possible to obtain nearly identical spider silk protein more efficiently and in greater quantities. These results also surpass RSSP creations through production in *Escherichia Coli* in terms of long strand creation (Kojima, Kuwana, Nakajima, Sezutsu & Tamada, 2014).

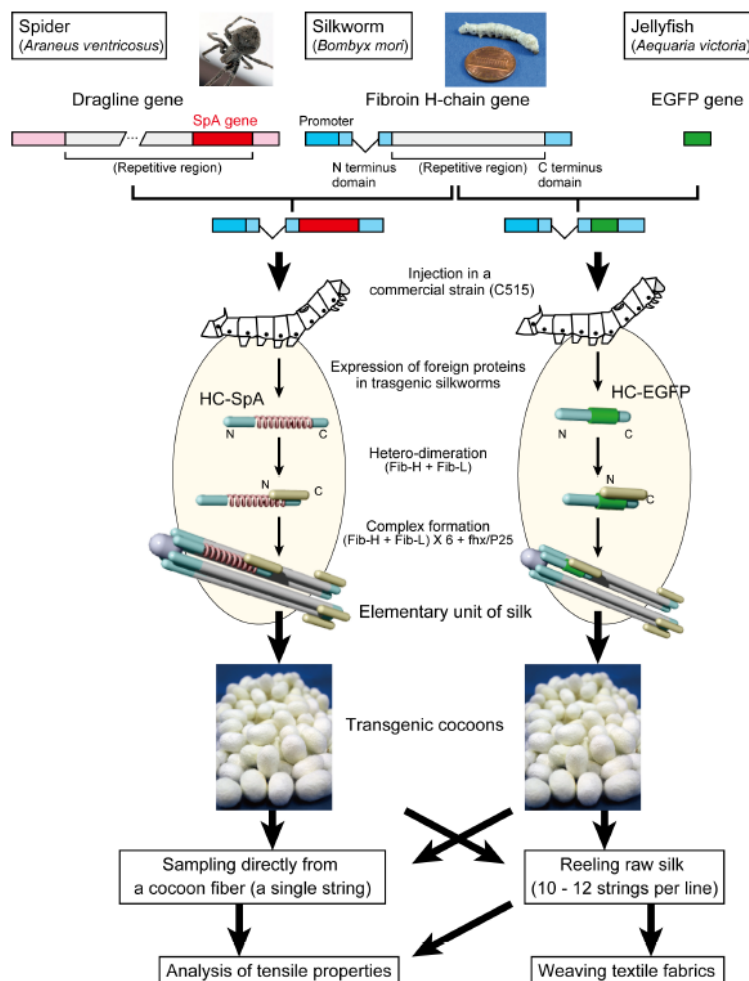


Fig. 4 Overview of the strategy used in this study. We cloned a partial sequence spider (*Araneus ventricosus*) dragline silk. The spider dragline protein (SpA) gene or enhanced green fluorescent protein (EGFP) gene were fused between the N- and C-terminal domains of the fibroin H-chain protein gene and the transgenic silkworm expressing these modified proteins, respectively. The transgenic silkworms expressed spider dragline protein or EGFP as a part of the silk fibroin proteins. The elementary units, consisting of fib-H, fib-L, and fhx/P25, were secreted into the lumen of the silk gland, and the silk was spun into a cocoon. The single cocoon silk and reeled raw silk were then prepared and their tensile properties analyzed. The raw silk was woven into yarn by an industrial machine to demonstrate its suitability for commercial applications. doi:10.1371/journal.pone.0105325.g001

(Kuwana et al., 2014)

These results indicate that it is possible to produce higher quality RSSP from transgenic animals with near natural physiological properties. This is encouraging as it is another avenue of experimentation towards practical applications and possible large scale production.

Creation and possible applications of RSSP were also noted by Schacht & Scheibel (2014). According to their article, Prokaryotic and Eukaryotic methods have been pursued to create a variety of morphologies such as films, hydrogels, particles, or non-woven meshes (Figure 5). Genetic modification allows for the incorporation of individual amino acid residues with chemically specific side-chains. The result is a broad range of applications through the covalent coupling of peptides, enzymes, or particles. Functionalizing RSSP with antimicrobial peptides is a step towards suppressing infections while promoting cell growth. Cell penetrating peptides can also be used for gene therapy. Coatings can be created with RSSP that boosts biocompatibility in materials such as medical grade silicone implants. Research shows that such coatings reduce inflammation and rejection. RSSP can also be encapsulated for drugs, proteins, genes, and cells for delivery or diagnostics (Figure 6). This makes them excellent drug carriers. They also show promise as gene carriers for tumor cell-specific delivery. In addition, use of mesh constructions showed improved ability of structures to regenerate and heal. For example, RSSP scaffolds were used to test cell repair (Schacht & Scheibel, 2014).

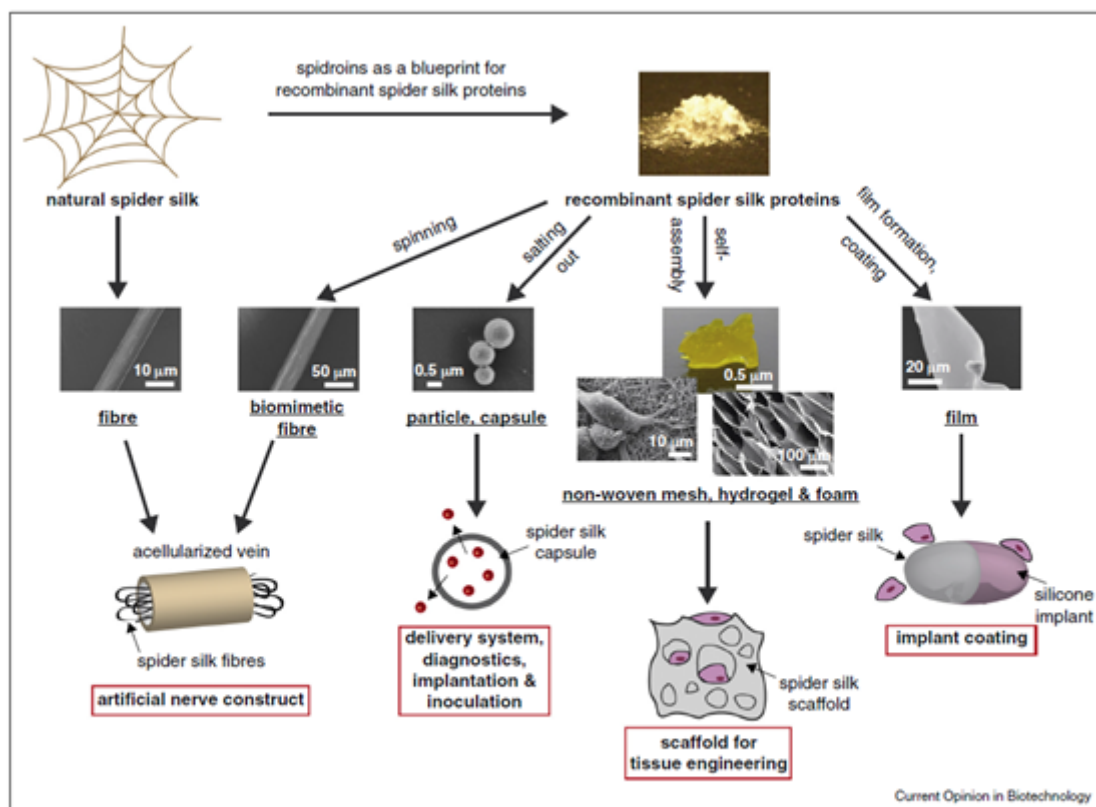


Fig. 5
Possible applications of spider silk materials in biomedicine. Recombinant spider silk proteins can be processed into morphologies other than fibres, broadening the spectrum of possible applications.

(Schacht & Scheibel, 2014)

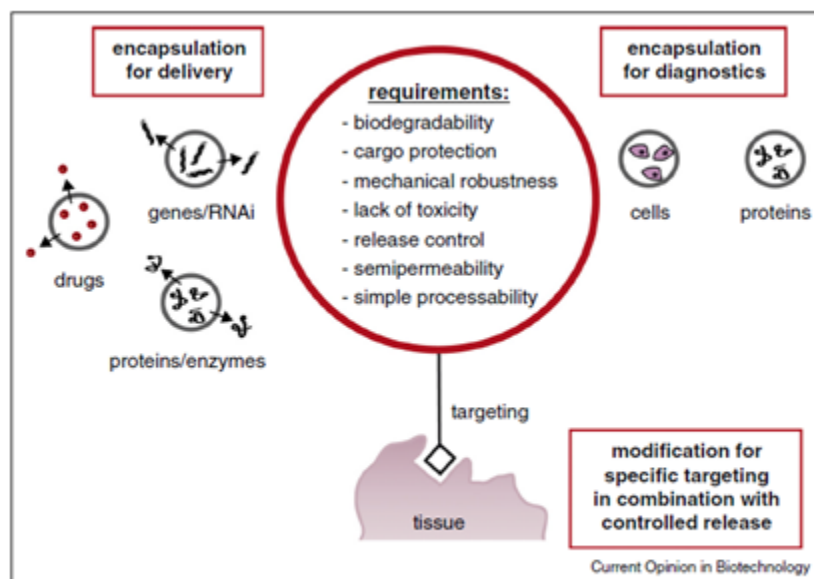


Fig. 6

Processing of recombinant spider silk proteins into colloidal systems for various biomaterials applications, such as drug, gene or enzyme delivery or cell and protein encapsulation for diagnostics.

(Schacht & Scheibel, 2014)

Applications of spider silk are not limited to RSSP alone. Hennecke et al. (2013) used natural spider silk woven together to form medical sutures for possible use in tendon repair (Figure 7). Braiding and use of various silks from a variety of spiders to minimize variations in tensile strength and strand diameters was involved during experimentation. Results showed increased tensile strength and positive reactions to NaCl for minimized retraction and physiological use. Cyclic tests of the braided spider silk strands far surpassed that of existing medical sutures and do not suffer the effects of rejection and infection (Hennecke et al., 2013).

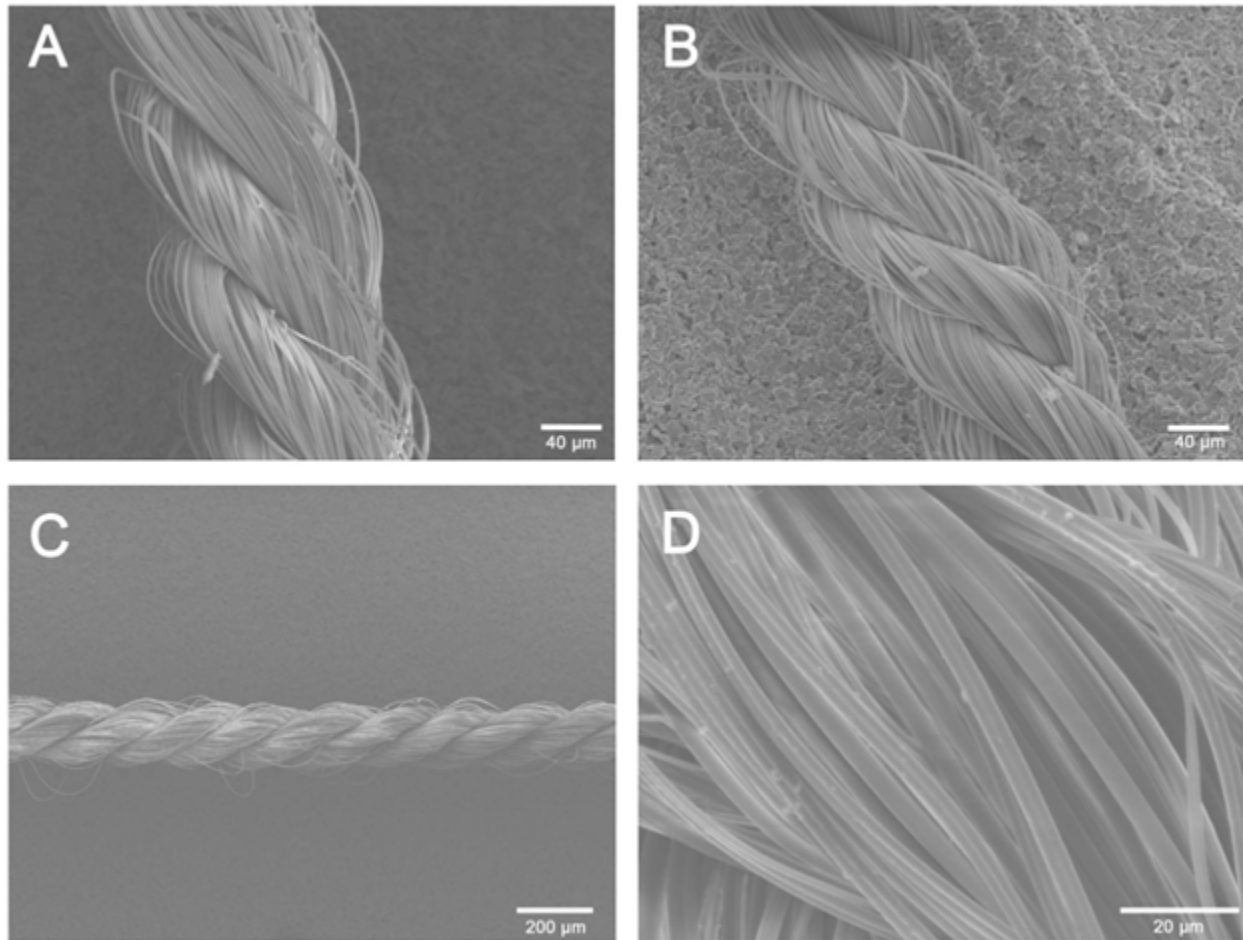


Fig. 7

(Hennecke et al., 2013)

Future possibilities of this research may lead to vastly improved clothing and textiles. Examples include materials that are stronger than steel and Kevlar but are supple, stretchable, and biodegradable. The secrets behind spider silk proteins can even lead to construction materials that replace existing ones with superior strength and protection. For instance, cabling and tubing made of RSSP coatings would be stronger than steel but be able to stretch and flex. Also, bridges supported by RSSP composed materials built into the support cables would be extremely versatile.

Advantages of this research into spider silk proteins include medical and commercial applications through RSSP with spider silk protein as a template. Targeted drug delivery, tissue repair, enhanced cosmetics, and improved clothing are all possibilities brought about by this research. In addition, use of natural spider silk or spider silk from transgenic animals can enable the production of a wide variety of materials that are stronger, biodegradable, and biocompatible. Some disadvantages of this research include: inhomogeneity in some RSSP's; difficulty in obtaining natural spider silk; differences in reactions of RSSP and spider silk due to temperature, environment, and silk type; differences in the protein structure of the spider silk among the many species of spiders; and the long term studies required to bring these technologies to market.

The ethical concerns surrounding the research and marketing of biotech products derived from RSSP and natural spider silk revolve around the testing done on animals during experimentation. We must ask ourselves if this is ethical. While much experimentation is still conducted on animals such as mice and rats for a variety of experiments, it is not something that

every individual feels is ethical. The research into spider silk proteins will definitely require experimentation on animals. However, I personally have no objection to this method. With that in mind, I still must consider the ethical concerns presented by others who may not share my view.

In conclusion, spider silk is an incredible material whose mechanical properties are ideal for the pursuit of biomaterials. It is biocompatible, biodegradable, has high tensile strength, and great elasticity. Biotechnological applications being experimented with currently can lead to targeted drug delivery, tissue repair, enhanced cosmetics, and improved clothing. Many future applications are also possible such as biomaterials like tough clothing, armor, and construction materials.

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