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Spider Silk Composites and Applications

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1. Introduction

In the past few years a number of reviews, books and journal articles featuring silks have been published. Silks are produced by over 41,000 species of spiders (class Arachnida) and by many insects (terrestrial and aquatic), particularly in the order Lepidoptera (Foelix, 1996); they are defined as externally spun fibrous material generated from protein secretions. The cocoon silk from the domesticated silkworm, *Bombxy mori*, represents one of the best characterized silks. Cocoon silks are natural composite materials that contain two core silk proteins, along with an outer adhesive protein dubbed sericin. Sericin can be removed by heat and alkaline treatment, leading to about 300 to 1,200 meters of usable fiber from each cocoon. Silk from spiders have also generated considerable scientific potential in recent years. Over the past two decades, scientists have been attempting to unravel the molecular details of spider silks because these composite materials offer a broader range of diverse mechanical properties. Spider silk fibers have remarkable mechanical properties and certain threads are superior to Nylon, Kevlar and high-tensile steel. As advances in the processes for their synthesis, processing, and extrusion continue to emerge it presents scientists with exciting opportunities for developing new classes of biomaterials. Spider silk proteins are synthesized from specialized abdominal glands that function as biofactories to produce large quantities of silk fibroins; these fibroins are spun into silks with different properties, compositions and morphologies. Spider silks have been reported to be used in the South Pacific for gill and dip nets, fishing lures, and in weaving ceremonial dresses. They have also been used clinically as sutures for centuries due to their biocompatibility, slow degradability and high tensile strength. Silks also have been shown to have piezoelectric properties and stability over a range of temperatures (Ando et al., 1980). Although cocoon silk from silkworms has been harvested by the Chinese for over 5,000 years, the domestication of spiders for large-scale silk collection is impractical due to their cannibalistic nature. Thus, because the development of spider silk farms is improbable, the interest has shifted to the development of recombinant DNA methodologies to clone spider silk genes for expression in transgenic organisms. The cob weaver black widow spider, *Latrodectus hesperus*, along with the orb weaver, *Nephila clavipes*, are rapidly becoming model organisms for retrieving the genetic blueprints from spider silk gene family members for expression studies. The long-term goal of the silk community is to express and purify recombinant silk proteins for a wide-range of applications, including medicine, engineering and defense. Here we cover the following topics: the diversity of spider silks, the composition and mechanical properties of silks, and the natural silk extrusion pathway. By drawing upon these biochemical data and processes, we describe the development of spider silk fiber composites using truncated recombinant spider silk proteins blended with

regenerated dragline silks. The application of mixing regenerated spidroins with recombinantly expressed spider silk proteins to produce composite fibers has not been explored by the silk community and this book chapter investigates the aspect of spinning artificial spider silk fibers for biomimetics.

2. Diversity of spider silks

As scientists unravel the secrets of spider silk, it has become evident that a broad range of diverse fiber types has evolved to serve different ecological purposes. These threads have a host of different functions: locomotion, wrapping prey, protection of developing eggs and web construction. The structural diversity of spider silk fibers can be attributed to over 400 million years of natural selection. Anatomical studies of typical orb or cob-weavers reveal the presence of seven distinct silk-producing glands: the major and minor ampullate glands (locomotion and web frame), tubuliform (egg case silk), flagelliform (spiral capture silk), aciniform (prey wrapping silk), aggregate (sticky adhesives on spiral capture or gumfooted lines), and the pyriform glands (attachment disc and joining fibers) (Fig. 1). Molecular and biochemical analyses demonstrate that the different silk-producing glands are highly specialized, expressing specific members of the spider silk gene superfamily (Table 1). Through the evolution of different silk-producing glands, spiders can express gland-specific silk proteins with different sequences, generating a broad range of different fiber types with diverse functional attributes (Guerette et al., 1996).



Fig. 1. Microdissection of the silk-producing glands from the abdomen of *N. clavipes* indicates several specialized structures that manufacture silk fibroins. Numbers indicate each gland: 1 (top and bottom – major ampullate), 2 (flagelliform), 3 (minor ampullate), 4 (aggregate), 5 (aciniform), 6 (tubuliform) and 7 (pyriform).

2.1 Major ampullate silks

The major ampullate gland has been the principal focus for researchers investigating the natural silk production pathway. This bulb-shaped gland is found in pairs in spiders and is responsible for producing dragline silk (Fig. 1). Dragline silk is referred to as the ‘safety line’

of spiders and it is often spun in pairs and is accompanied with two minor ampullate silk threads (Fig. 2A). During locomotion, dragline silk fibers are immobilized to surfaces by structures known as attachment discs. Attachment discs are comprised of small diameter fibers spun from the pyriform gland that are extruded in a sticky substance that dries rapidly, sealing dragline silk to wood, concrete, glass, plants and other materials (Fig. 2B). The mechanical properties of major ampullate silk are dependent upon the amino acid sequences of the spun fibroins and the reeling speed during extrusion. When major ampullate silks are spun rapidly, it produces a stiffer fiber that efficiently supports the weight of spiders. In contrast, spinning major ampullate silk at a slower rate during the web frame construction leads to a fiber with higher extensibility, a necessity for dissipating the energy of prey impact on the web. Transmission electron microscopic analysis reveals dragline silk contains four different layers: core, skin, glycoprotein layer and a lipid coat (Sponner et al., 2007). These studies also demonstrate that dragline silk threads lack adhesive coatings on their surface.

Spider Silks and their Natural Functions in Cob weavers

<u>Silk Gland</u>	<u>Location of Silk</u>	<u>Fiber Proteins</u>
Major ampullate	Web frame, safety line (dragline)	MaSp1; MaSp2
Minor ampullate	Web reinforcement , safety line (dragline)	MiSp1-like
Flagelliform	Spiral capture silk*	Flag*
Aciniform	Wrapping silk, small diameter egg case fiber	AcSp1-like
Tubuliform	Large diameter egg case fiber	TuSp1; ECP-1; ECP-2
Aggregate	Glue coating for gumfooted lines	Unknown
Pyriform	Attachment disk and joining fiber	PySp1

Table 1. Summary of silk-producing glands, fiber types and silk proteins found in the threads of orb- or cob-weavers. (*Only identified in orb-weaver's spiral capture silk)

In orb-weaving spiders the functions for dragline silk include web frame construction and locomotion. Black widow spiders also use dragline silk to build three-dimensional webs for prey capture. We have shown by MS/MS analyses that solubilized proteins from scaffolding, gumfooted and dragline silk fibers, followed by tryptic digestion, produce overlapping peptide fingerprints that match protein sequences within the major ampullate fibroins (unpublished data). These studies demonstrate scaffolding, gumfooted lines and dragline silk consists of major ampullate silks. Because scaffolding silk fibers represent the bulk of a black widow spider's three-dimensional web, it provides an excellent opportunity to collect dragline silk from black widow spiders housed in cages. The collection of large amounts of scaffolding silk can be done rapidly from spiders stored within the laboratory. Milligram quantities can be obtained from a single cage that is 1' x 1' x 1.5'. In order to produce regenerated major ampullate silk, we have taken advantage of the fact that scaffolding fibers are a rich source of full-length protein sequences for the major ampullate silk fibroins.

2.2 Egg sac silks

Of the seven different silk types spun from spiders, egg case silks are the easiest to collect. When egg sacs are spun from female spiders, they must endure a variety of different

environmental factors. Some of these factors include predators and invasion by parasites or temperature and humidity fluctuations. Black widow spider egg sacs contain two thread types: aciniform and tubuliform silks (Fig. 2C-D). Aciniform fibers have diameter sizes that are approximately 500 nm, which is one order of magnitude smaller than the average 5 μm diameter sizes for tubuliform silks. These fibers are also coated with small droplets, but the chemical contents of the liquid material have not been determined (Fig. 2C-D). In addition to the structural proteins in the fibers, MS/MS analysis has identified the presence of two spider coating peptides, which have been named SCP-1 and SCP-2. Intriguingly, these peptides can be efficiently removed from egg sacs soaked in water, suggesting these peptides are water soluble, polar compounds (Hu et al., 2007). The biological significance of the coating peptides is presently unknown, but one hypothesis is these molecules function as antimicrobial agents that help prolong the longevity of the fibers. Quantitative real-time PCR analysis has demonstrated that SCP mRNA levels are elevated in the flagelliform gland (Blasingame et al., 2009).

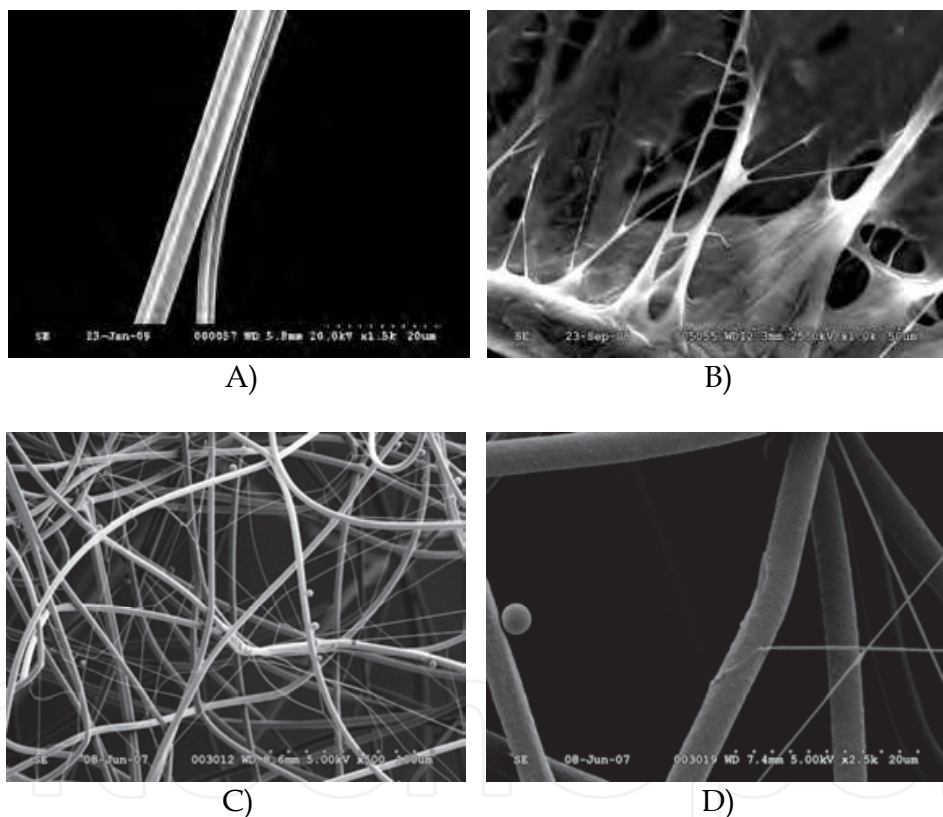


Fig. 2. Scanning electron microscopy of dragline silk, attachment disc fibers and egg sacs from the black widow spider, *L. hesperus*. A) Dragline silk; B) Attachment discs; C) Egg sacs at 500X magnification; D) Egg sacs at 2500X magnification.

In addition to aciniform silks being constituents of egg cases, these fibers are also used for prey capture (Hayashi et al., 2004; Vasanthavada et al., 2007). Because detailed molecular data are available for dragline and tubuliform silks and these fiber types are available in large quantities, we are attempting to study the mechanical behavior of synthetic composite spider silk threads spun from regenerated dragline and egg sac silk protein mixtures. Additionally, we are supplementing regenerated dragline silk fibroins with different

recombinantly expressed spider silk proteins. This approach has many advantages, including the ability to have native-sized full-length fibroins in artificially spun fibers with truncated recombinant proteins.

3. Properties and compositions of spider silk

Spider silks represent natural composite materials that are biopolymers. Biochemical analyses reveal that the different silk types contain at least 2-3 distinct structural proteins, commonly referred to as fibroins. Table 1 summarizes the major proteins stored within the luminal contents of several silk-producing glands within *L. hesperus*. With respect to molecular characterization, dragline silk has received the most attention from the scientific community.

3.1 Molecular components of dragline silk

The major and minor ampullate fibroins contain the highest levels of glycine and alanine relative to other spider silk family members; these levels approach >50% of the total amino acid content (Casem et al., 1999; Lombardi & Kaplan, 1990). Biochemical experiments show dragline silk is a composite material largely composed of two structural proteins or spidroins (contraction of the words spider and fibroin) called MaSp1 and MaSp2 (Xu and Lewis, 1990; Hinman and Lewis, 1991). Structural studies demonstrate the major ampullate spidroins form the core of the fiber that is wrapped inside a glycoprotein coat. Although the identities of the constituents of the glycoprotein layer remain unknown, experimental evidence supports this layer is added in the ampulla prior to extrusion (Casem et al., 2002; Sponner et al., 2005). The molecular sequences coding for the dragline silk fibroins were the first to be identified from *N. clavipes* (Xu & Lewis, 1990). Recently, the complete genetic blueprints for MaSp1 and MaSp2 were determined from *L. hesperus* (Ayoub et al., 2007). The predicted sequences for these fibroins encode large molecular weight proteins that are approximately 3,500 amino acids in length. These spidroins are highly modular, each containing internal repetitive block repeats that are flanked by N- and C-terminal non-repetitive ends comprised of approximately 100 amino acids. The internal block repeats are rich in glycine and alanine; these regions form polyalanine or polyalanine-glycine stretches that are interrupted by glycine-rich regions. The polyalanine segments form beta-sheet crystal domains and are responsible for the high tensile strength while the glycine-rich regions adopt 3_1 -helix type structures and beta-turns that link the crystalline domains (Simmons et al., 1996). These interconnecting glycine-rich regions constitute the semi-amorphous regions and have been implicated in the extensibility of the fibers. Extensibility of dragline silk fibers also has been attributed to glycine-proline-glycine-X-X (GPGXX) repeats within the MaSp2 protein sequence and the formation of beta-spirals. MaSp2 proteins have been shown to be tightly packed in certain core regions of fibers from *N. clavipes*, whereas MaSp1 appears to be uniformly distributed along the radial axis (Sponner et al., 2005). These data demonstrate that MaSp1 and MaSp2 are not evenly distributed down the long axis of natural fibers. The biochemical mechanisms that modulate their differential localization are not well understood, but could be explained by differences in expression levels and/or their protein sequences that control partitioning during extrusion. Interestingly, in natural fibers, MaSp1 and MaSp2 ratios have been shown to vary between different species (Tso et al., 2005); these differences have been linked to the diet and environment of spiders (Craig et al., 2000). It has been suggested that the synthesis of MaSp2 is more energetically expensive because of the higher cost associated with proline

biosynthesis. Therefore, it would appear that spiders synthesize cheap silk when resources are limiting, perhaps producing fibers that contain predominantly MaSp1 molecules. By testing the mechanical properties of composite spider silk fibers spun from regenerated dragline silk fibroins combined with different ratios of recombinant MaSp1 and MaSp2, it will help address the structural roles of the MaSp1 and MaSp2 fibroins. It will also reveal how this spider silk composite behaves from a mechanical perspective. To a large extent, this has not been fully explored because obtaining large amounts of purified recombinant silk proteins for the spinning process is a difficult task.

3.2 Molecular components of tubuliform silks

Tubuliform fibroins have been shown to contain considerably lower levels of glycine relative to dragline silk. Similar to pyriform silks, tubuliform silks incorporate amino acids with more polar side chain groups (Blasingame et al., 2009). Although dragline silk fibers have received much attention in the scientific community, the molecular and structural details for egg case silk fibers are rapidly emerging. Egg sacs are produced by female spiders during reproductive periods. For black widow spiders, egg cases are composite materials containing two different silk types: tubuliform and aciniform (Vasanthavada et al., 2007). The presence of two different fiber types implies tubuliform and aciniform fibers provide a unique role that is central to the protection of spiderlings during development. Biochemical studies have shown that aciniform silks contain the fibroin AcSp1-like (Vasanthavada et al., 2007) and the tubuliform silks contain at least three different proteins: TuSp1, ECP-1 and ECP-2 (Hu et al., 2005; 2006b). Scanning electron microscopy, along with amino acid composition analyses performed on the egg sacs, support that tubuliform silks, based upon volume, constitute the bulk of egg sac fibers. Additionally, mass spectrometry studies (MS/MS) demonstrate that TuSp1 is one of the major constituents of tubuliform silks and the non-repetitive N-terminus is retained in the spun fibroin. Manual inspection of the amino acid sequence of TuSp1 reveals high levels of serine and alanine, but lower levels of glycine. This fibroin lacks the traditional A_n , $(GA)_n$, $(GGX)_n$ and $(GPGXX)_n$ repeats, but it contains new amino acid motifs that include S_n , $(SA)_n$, $(SQ)_n$ and GX (X represents A, D, F, I, N, Q, V and Y). Internal block repeats are ~184 amino acids in length and are extremely homogenous in nature. Short polyalanine stretches are highly iterated in the TuSp1 sequence and ^{13}C NMR spectroscopy demonstrates that the majority of alanine is in a beta-sheet structure in post-spun egg case silk (Hu et al., 2005).

The ECPs (ECP-1 and ECP-2) are also components of tubuliform silks (Hu et al., 2006b). Based on Q-PCR studies, TuSp1 mRNA levels are ~20-fold higher relative to the ECPs. Both ECP-1 and ECP-2 contain large amounts of alanine, glycine and serine. These residues account for more than 50% of all of the amino acids in their translated sequences. Translation of the ECP-1 and ECP-2 cDNAs predict proteins with 932 and 825 amino acids, respectively. The N-terminal 182 residues of ECP-1 and ECP-2 are 70% identical, with 16 cysteine residues showing identical spacing patterns and 100% conservation. Whether these cysteines participate in disulfide bond formation with each other or the N-termini of TuSp1, which has two cysteine residues within its non-repetitive conserved terminus, is unknown. The ECPs lack traditional internal block repeats commonly found in spidroin family members, however, ECP-2 contains scattered, internal blocks of polyalanine and a C-terminus rich in GA repeats. To date, the orb-weaver ECP orthologues have not been reported. The precise role the ECPs serve in tubuliform silks is unclear, but their rich alanine and glycine content, similar to dragline silk fibroins, suggests structural importance.

4. Mechanical properties of spider silk

The mechanical properties of spider silks have been shown to be highly renowned relative to some of the best man-made materials, displaying a combination of high strength, extensibility and toughness. With respect to energy absorption prior to breaking, spider silks are unmatched in the world of synthetic and natural fibers (Table 2). Different spider silk types produce distinct stress-strain curves, demonstrating that spiders spin a broad range of fibers with diverse mechanical properties (Gosline et al., 1986; Hu et al., 2006a). Similar to other protein polymers, there are numerous factors that influence the strength and the load-deformation response of silk fibers, which includes temperature, humidity, and extrusion rate. The majority of mechanical studies have focused on dragline silk. Dragline silk fibers have been subject to tensile testing from a wide range of different spider species. Natural dragline silk fibers show both extremely high tensile strength and toughness (often measured as “Energy to Break”). In man-made polymers, such as polyethylene, increasing strength would often compromise fracture toughness. Yet in the dragline silk, both strength and toughness can be maintained at high levels. The variations in the mechanical behavior in dragline silk fibers across different species suggest that these silks have been fine-tuned by each spider for their nuanced needs. Other spider silk fiber types have also been studied using tensile testing, including aciniform silks (Hayashi et al., 2004), tubuliform silks (Hu et al., 2006a), flagelliform silks (Lewis, 2006) and minor ampullate silks (Liivak et al., 1997).

Fiber	Elongation (%)	Strength (GPa)	Energy to Break (MJ/m ³)
<i>Araneus</i> dragline	27	1.1	160
<i>L. hesperus</i> dragline	34	1	nd
<i>B. mori</i> cocoon silk	18	0.6	150
Flagelliform	>200	1	150
Minor ampullate	30	0.346	nd
Tubuliform	71.7	0.629	nd
Aciniform	80	0.7	290
Nylon fiber	18	0.95	80
Kevlar 49 fiber	2.7	3.6	50
High-tensile steel	0.8	1.5	6

*Data was collected from several sources: Gosline et al. 1999; Hayashi et al., 2004; Livak et al., 1997; Lawrence et al., 2004; Hu et al. 2006a. nd = no data

Table 2. Mechanical properties of spider silks in comparison to other fibers.

4.1 Dragline silk

Dragline silk is five times stronger by weight than steel and three times tougher than the man-made fiber Kevlar, a synthetic fiber used in body armor (Table 2). Correlations between the reeling speed and the tensile strength of dragline silk fibers have been observed experimentally, supporting that spiders can tailor their thread properties by adjusting the draw rate from their spinnerets. By controlling the reeling speeds during extrusion, spiders can tune beta-sheet crystallite size and orientation of the protein polypeptide chain network. These parameters are important structural factors that modulate the tensile strength of silks (Du et al., 2006). Studies investigating the effects of reeling speeds demonstrate 3 nm nanocrystals are optimal for producing fibers that have exceptionally high tensile strength.

Consistent with this observation, molecular modeling stress-strain simulations predict that 3 nm crystals, which correlate to the natural size found in dragline silk, have higher ultimate strengths and toughness relative to fibers with larger nanocrystal sizes (Nova et al., 2010). This implies that spiders can manipulate the material properties of their fibers by changing the reeling speed to tune the size of the beta-sheet nanocrystals.

Spider dragline silk fibers have been shown to be thermally stable to about 230°C on thermal gravimetric analysis. In addition, these fibers have been shown to be able retain their morphology, structure and mechanical properties when autoclaved (Hedhammar et al., 2010). Unlike tubuliform silks, natural dragline silk fibers have been shown to experience supercontraction when exposed to water, resulting in shrinking by up to 50% in length. For certain silk types, supercontraction may provide an important mechanism to tailor silk properties for synthetic composite spider silks.

4.2 Minor, aciniform and flagelliform silk

Similar to major ampullate silks, minor ampullate silks have been shown to display a high Young's modulus (Liivak et al., 1997). In addition, mechanical studies have demonstrated that aciniform silks represent the toughest spider silk type (Hayashi et al., 2004), a likely evolutionary adaptation to help prevent the escape of immobilized prey (Table 2). Previous biochemical analyses performed by our laboratory have demonstrated that aciniform silks are also constituents of egg case silk in black widow spiders. The presence of aciniform silks in egg sacs is intriguing, but it may suggest these fibers function to prevent predators from gaining access to the enclosed eggs. Flagelliform silks, which have only been studied from orb-weavers, are highly extensible fibers that constitute spiral capture silk (Lewis, 2006). Spiral capture silk fibers are strategically positioned in orb webs to absorb and dissipate large amounts of mechanical energy introduced during the collision of aerial insects (Lin et al., 1995). These fibers are extremely compliant, which results in energy dissipation through thread displacement and stretching (Table 2). Whether a functional equivalent of spiral capture silk fibers is present in black widow spiders is unclear. Black widow spiders cast three-dimensional webs, instead of two-dimensional webs that are characteristic of orb weavers. Therefore, it would appear that black widow spiders have replaced FLAG silk, which comprises the spiral capture silk fibers, with scaffolding silk or dragline silk. This observation supports that spiders have evolved specialized silk fibers and different web types to optimize prey capture.

4.3 Tubuliform silks

Tubuliform silks have been shown to be highly extensible, but display lower tensile breaking strength relative to dragline silk fibers (Hu et al. 2006a; Zhao et al., 2006). However, because tubuliform silks have increased extensibility relative to dragline silks, tubuliform fibers display similar toughness (Table 2). To our knowledge, no experiments have been reported to examine the mechanical properties of regenerated tubuliform silk.

5. Natural extrusion of silk

Many laboratories have focused on studying the extrusion process of dragline silk from the major ampullate gland, primarily because this tissue is the easiest to identify and remove. This structure is also the largest relative to the other silk-producing glands. Morphological and histological studies demonstrate the major ampullate gland consists of various components: it has a tail, ampulla, funnel and extrusion duct (Fig. 3).

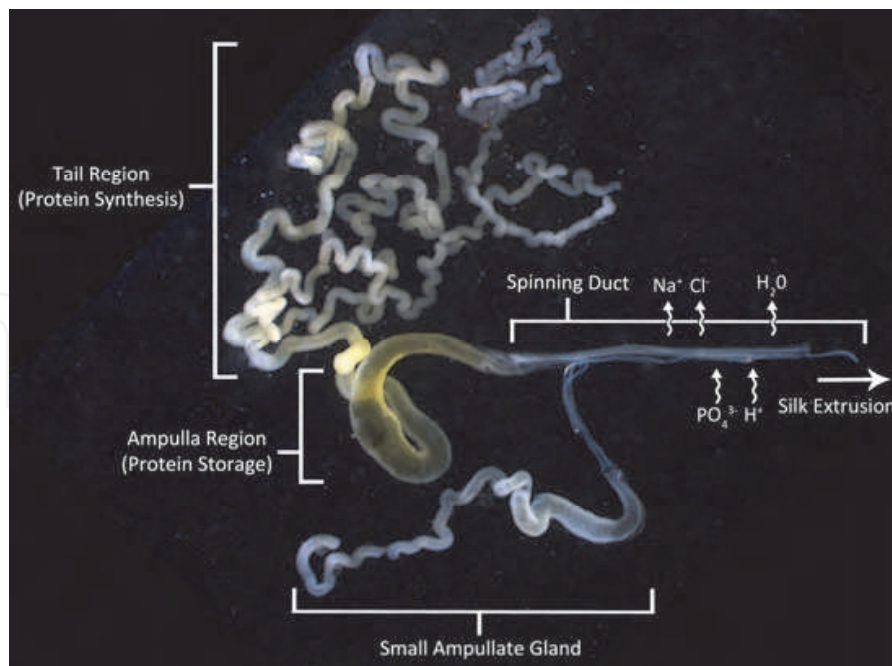


Fig. 3. The major ampullate gland of *N. clavipes* with tail, ampulla and the extrusion duct (spinning duct). Shown connected is the small ampullate gland. Biochemical events known to occur during the extrusion process are also labeled.

Large quantities of major ampullate spidroins are manufactured from columnar epithelial cells residing in the tail, transported to the ampulla through vesicles, followed by extrusion through a duct that tapers down, finally exiting from a spigot positioned on an abdominal spinneret. In our microdissections of spiders, we also observe the presence of a small ampullate gland (SAG) that is connected to the major ampullate gland (Fig. 3) (Ortiz et al., 2000). The SAG is distinct from the minor ampullate gland (not shown) and it has received little attention in scientific literature. Intriguingly, the SAG has its own tail, ampulla, funnel and spinning duct that is fused to the extrusion duct of the major ampullate gland (Fig. 3). The specific biological function of the SAG or the molecular contents it synthesizes, stores and spins is unclear.

5.1 Biochemical processes during silk extrusion

To be able to produce synthetic spider silk composite fibers, scientists must first understand the natural spinning process. Biophysical studies of the liquid contents stored within the major ampulla, also referred to as the spinning dope, reveal these proteins are unfolded and have a disordered secondary structure (Knight et al., 2000; Lefevre et al., 2008). The spinning dope has been shown to represent a highly concentrated aqueous mixture of major ampullate spidroins. During extrusion, these spidroins undergo changes that alter their conformation and orientation. This process, which involves converting native liquid silk into insoluble fibers, is modulated by chemical and mechanical events (Fig. 4).

These events include changes in pH, salt concentration and elongational flow (Chen et al., 2002; Dicko et al. 2004; Knight & Vollrath, 2001). This course of action gives rise to a hierarchically organized semicrystalline material. The crystalline region consists of short polyalanine segments that form beta-sheet nanocrystals, whereas the amorphous phase is formed from glycine-rich segments that likely adopt 3_1 -helices and a few alpha helices.

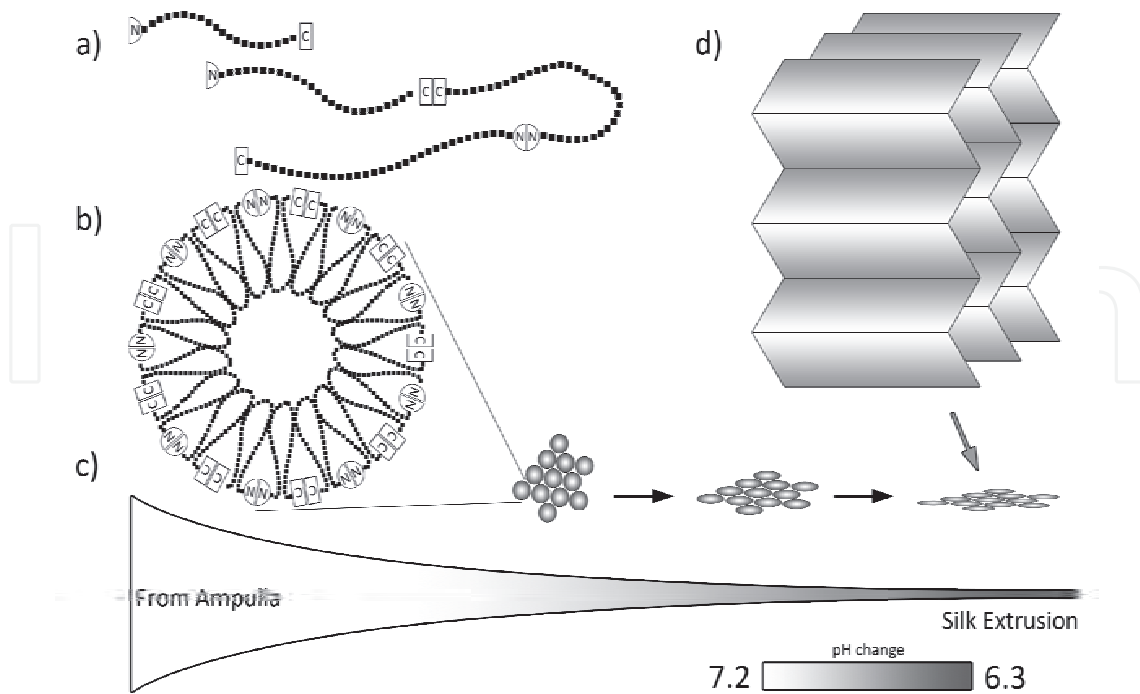


Fig. 4. Biochemical events that occur during the silk extrusion pathway (a-d). a) monomers assembling into multimers via their N- and C-termini; b) micelle and globule-like structure formation; c) fibroin concentration and hydrogen ion levels increase (left to right) as the proteins transition from a liquid to solid state, traveling from the ampulla through the spinning duct; d) Beta-sheet structure of crystallite regions.

Histological data reveal that H^+ pump activity increases steadily along the length of the spinning duct (Vollrath et al., 1998). Studies using a pH-sensitive microelectrode probe support this observation, demonstrating the pH decreases from 7.2 in the major ampullate gland to 6.3 in the extrusion duct (Dicko et al., 2004). Other chemical changes have been implicated in the transition from liquid to solid during fiber production. These include a decrease in Na^+ ions, coupled with increased K^+ and phosphate ion concentrations during extrusion (Huemmerich et al., 2004) (Fig. 3). The pH decrease in the extrusion duct has been associated with changes in the ionization state of side chain residues positioned within the conserved N- and C-termini of spidroins, promoting dimerization and fiber polymerization. Although the N- and C-termini both contain conserved non-repetitive regions, their amino acid sequences are dissimilar. However, despite the differences in amino acid sequences, there are some similarities in their conformational flexibility and secondary structures. Recent structural studies of the MaSp1 N-terminal domain (NT) from the nursery web spider, *Euprosthenops australis*, demonstrate this region mediates protein assembly (Askarieh et al., 2010; Hagn et al., 2010). Using recombinant NT mini-spidroins, it has been shown that charge-dependent self-assembly occurs at pH values around 6.3 and promotes dimeric formation, whereas at pH values around 7, the NT domain delays protein aggregation and the spidroins remain in a monomeric state. Although pH changes have been implicated in the assembly process, it is unclear whether other non-fibroin proteins also participate in enhancing fibroin solubility or folding. Peroxidases have been detected in the luminal contents of the major ampulla, but their biological function and contribution to fiber assembly has not been established (Pouchkina et al., 2003). Future studies of the glandular

contents using mass spectrometry, combined with transcriptome analyses of the different silk-producing glands, should help lead to the identification of new molecular components that modulate the assembly process. These findings will undoubtedly have a substantial impact on manufacturing strategies that integrate silk proteins in composite materials.

The mechanisms of fiber extrusion in the other silk-producing glands have not been thoroughly investigated at the biochemical level. Analyses of the morphological features of these silk-producing glands reveal profound structural differences, aside from the architecture of the minor ampullate gland, which closely resembles the major ampullate gland (Jeffery et al., 2011). These data imply that the mechanisms for fiber extrusion are distinct in the different glands that produce silk. Raman spectroscopy studies performed on the glandular contents of the tubuliform gland support this supposition, which demonstrate proteins are stored in a globular conformation with a high degree of alpha helical nature; this differs significantly from the conformations of fibroins stored within the major ampullate gland (Lefevre et al., 2011). However, after spinning, the tubuliform silks are structurally similar to major ampullate fibers, containing a high degree of beta-sheet structure. NMR studies have been performed to analyze the secondary and tertiary structures of *N. antipodiana* TuSp1. Recombinant proteins containing either the N- or C-termini attached to one repetitive domain spontaneously assemble into micelle-like structures that can be converted into fibers at $\geq 37^{\circ}\text{C}$ (Lin et al., 2009). The N- and C-termini, which are less hydrophilic relative to the internal block repeats, assemble and form the outer region of the micelles, whereas the internal block repeats become buried on the inside of the micelles; these internal block repeats exist in an unfolded state (Lin et al., 2009). During extrusion the concentration of silk fibroin in the gland gradually increases to form micelles, which further form globule-like structures and gels. These structures then experience shear force, resulting in the conversion of random coil protein structures into beta-sheet structures. Solid state NMR conducted on egg case silk fibers is consistent with this transition and supports the presence of beta-sheet structures in spun fibers.

6. Synthetic composite spider fibers from recombinant proteins

Although it is feasible to harvest milligrams of natural dragline silk by forcibly “milking” spiders or retrieving large amounts of tubuliform silks through the collection of egg sacs, these are expensive and time-consuming endeavors. For example, a 3.4 m textile weaved from natural dragline silk took more than one million *N. madagascarenis* spiders and the efforts of over 70 individuals working 4 years; the total cost exceeded over \$500,000. Moreover, attempts to farm spiders have proven unsuccessful given their intrinsic cannibalistic nature. Because of these difficulties, scientists have turned to using recombinant DNA technologies to express spider silk proteins in transgenic systems. Expression of silk proteins in heterologous systems has much promise; however, several challenges still need to be resolved before artificial silk fibers can be spun from recombinant proteins on an industrial scale. These include the development expression systems that manufacture large quantities of full-length recombinant silk protein in a fast, cost effective manner, and the second involves the development of spinning apparatuses that produce silk fibers with mechanical properties approaching natural threads, preferably without the accumulation of toxic waste products during the manufacturing process. Since harvesting large quantities of silk from domesticated spiders for industrial applications is impractical, recombinant spider silk production in either prokaryotic and/or eukaryotic organisms will

need to be developed to ensure large amounts of soluble protein are available for artificial fiber production.

6.1 Silk fibroin expression

Both prokaryotic and eukaryotic expression systems have their advantages and disadvantages. For example, many eukaryotic proteins require posttranslational modifications for functional activity, such as glycosylation and phosphorylation. Although the significance of posttranslational modifications (PTMs) to fibroins remains unclear, amino acid residues have been shown to be subject to phosphorylation and glycosylation within fibroin sequences (Michal et al., 1996). Because PTMs are largely restricted to eukaryotic cells, expression systems equipped with the cellular machinery for PTMs offer benefits to potentially achieving fibroin states that resemble *in vivo* conditions. There have been several successful reports of partial or truncated fibroins expressed in prokaryotes, in particular *Escherichia coli*. However, the expression of full-length fibroin cDNAs have not been reported in either prokaryotic or eukaryotic expression systems. Both expression and replication of spider silk cDNAs has been hampered by their long, repetitive and guanine-cytosine-rich nature. These intrinsic properties have presented technical challenges with sequencing spider silk genes, the ability to maintain intact transcription units for expression studies, and the translation of long mRNAs dominated by alanine and glycine codons. The inability to express full-length fibroins has been attributed, in part, to the depletion of the tRNA pools, low solubility of the fibroins during expression and proteolysis of the recombinant silk proteins. Several different cloning strategies have been applied to overcome these barriers, including codon optimization of silk genes, specialized growth media and conditions, as well as use of a variety of different host organisms for recombinant silk production (Fahnestock and Irwin, 1997; Schmidt et al., 2007). Various plants have been explored as model systems for spider silk expression, including *Arabidopsis* (Yang et al., 2005), tobacco and potato (Scheller et al., 2001). Mammalian cell culture systems have shown some promising success for expressing large molecular weight fibroins using the immortalized cell lines from bovine mammary epithelial alveolar cells and baby hamster kidney cells (Lazaris et al., 2002). Transgenic mice and goats that express spidroins also have been generated, but these animals have resulted in the expression of low levels of fibroins and have not proved to be cost effective for large-scale production of silk proteins (Xu et al., 2007). Efforts to use the methylotrophic yeast *Pichia pastoris*, a robust eukaryotic expression system that allows for recombinant proteins to be secreted into liquid growth media, have also been explored but with limited success (Fahnestock & Bedzyk, 1997). Perhaps the most success for producing recombinant spider silk proteins that approach native sizes has come from the development of a metabolically engineered *E. coli* strain (Xia et al., 2010). However, despite this most promising case, the levels of expressed proteins that can be readily purified for the spinning process still remain too low for industrial scale production.

6.2 Artificial fiber production

Despite the broad range of expression systems used to synthesize recombinant spider silk proteins, only a handful of research groups have spun artificial silk fibers using the recombinant spidroins (Geurts et al., 2010; Lazaris et al., 2002; Teule et al., 2009). Even fewer research labs have subsequently used these synthetic fibers to compare their mechanical properties to the natural fibers (Xia et al., 2010). For most labs that have participated in the production of recombinant silk fibroins, strategies have included using several block repeats

and/or the non-repetitive C-terminal domain for expression studies. The majority of these studies have used either the major ampullate spidroin 1 or 2 (MaSp1 or MaSp2). *Araneus diadematus* factor 3 and 4 (ADF-3 and ADF-4), two variants of MaSp2, have been used in a number of studies. Recombinant MaSp1 spidroins have been produced using natural cDNA sequences or multimerized synthetic gene pieces assembled from oligonucleotides (Fahnestock & Bedzyk, 1997). In both cases overexpression of the truncated recombinant proteins has often resulted in products that are water insoluble or precipitate out of solution during protein purification. Several different strategies have been attempted to increase the fibroin solubility during expression. One approach has been to express fusion proteins that contain N- or C-terminal solubility-enhancing fusion partners such as thioredoxin (Stark et al., 2007). Other strategies have included the incorporation of enzymatic phosphorylation/dephosphorylation switches (Szela et al., 2000; Winkler et al., 2000) or the integration of oxidation/reduction residues into the fibroin amino acid sequence to control the assembly process (Winkler et al., 1999). Although these methods have led to improved solubility, premature aggregate formation has been difficult to control during expression and protein purification. Currently, solubilization of aggregates is often achieved using hexafluoroisopropanol (HFIP), urea, guanidine hydrochloride, LiBr or formic acid. Treatment of the aggregates with these solvents has become necessary to obtain sufficient amounts of solubilized recombinant silk proteins for the artificial spinning process, which requires 15-50% of spinning dope. However, because these solvents are hazardous and removal of them from the spun threads is challenging, alternative approaches that integrate spinning fibers from a water-based solvent will need to be developed and applied to ensure the successful use of fibers for biomimetics.

6.2.1 Composite silk fibers spun from regenerated major ampullate silk spidroins blended with recombinant proteins

Although synthetic silk fibers have been spun from recombinant spider silk proteins, silk filaments have also been reported from dissolving natural dragline silk and re-spinning the solubilized fibroin extract, a process that leads to a material known as regenerated silk. Regenerated dragline spider silk has been described in the scientific literature just a few times. These experiments have demonstrated reconstituted dragline spider silk self-assembles into filaments from *N. edulis* (Shao et al., 2003). Additionally, these fibers were shown to contain similar amino acid compositions relative to natural silks, suggesting the molecular assembly process could be mimicked *in vitro*. For these studies, the investigators focused on gleaning the molecular details about the spinning process.

In our attempt to enhance the properties of regenerated dragline spider silk, we have supplemented this silk with recombinantly expressed truncated dragline silk fibroins, MaSp1 and MaSp2. These truncated forms, referred to as 1RCT_{MaSp1} and 1RCT_{MaSp2}, consisted of one block repeat fused to the non-repetitive, conserved C-terminus. To facilitate recombinant protein solubility during induction in *E. coli*, we added an N-terminal thioredoxin tag. Recombinant proteins were purified by using a 6x-his tag placed on the C-terminus. Following purification, different ratios of 1RCT_{MaSp1} and 1RCT_{MaSp2} were combined with regenerated scaffolding silk fibroins and spun into synthetic fibers. To obtain regenerated fibroins, scaffolding fibers were collected from cages and dissolved overnight in hexafluoroisopropanol (HFIP) at a final concentration 15% (w/v). HFIP is a commonly used spinning solvent. Reconstituted scaffolding fibers and blended filaments were spun using a previously published protocol (Teule et al., 2009). Mixing different ratios of the recombinant

proteins led to reconstituted composite fibers that morphologically resembled fibers spun from regenerated spider silk alone (Fig. 5A-D). These fibers had a fibrillar structure and contained diameter sizes ranging from 33-49 μm . The morphology of the regenerated as well as the blended fibers was also similar to native dragline silk fibers (Compare Fig. 2A with 5A-D). We also examined the fiber interior core after breaking the threads. Analyses of the fractured regenerated fibers showed a solid, smooth interior (Fig. 5E) whereas the blended silk fibers were more fibrous-like (Fig. 5F). The more fibrous-like interior may be the result of fibroin assembly from truncated proteins.

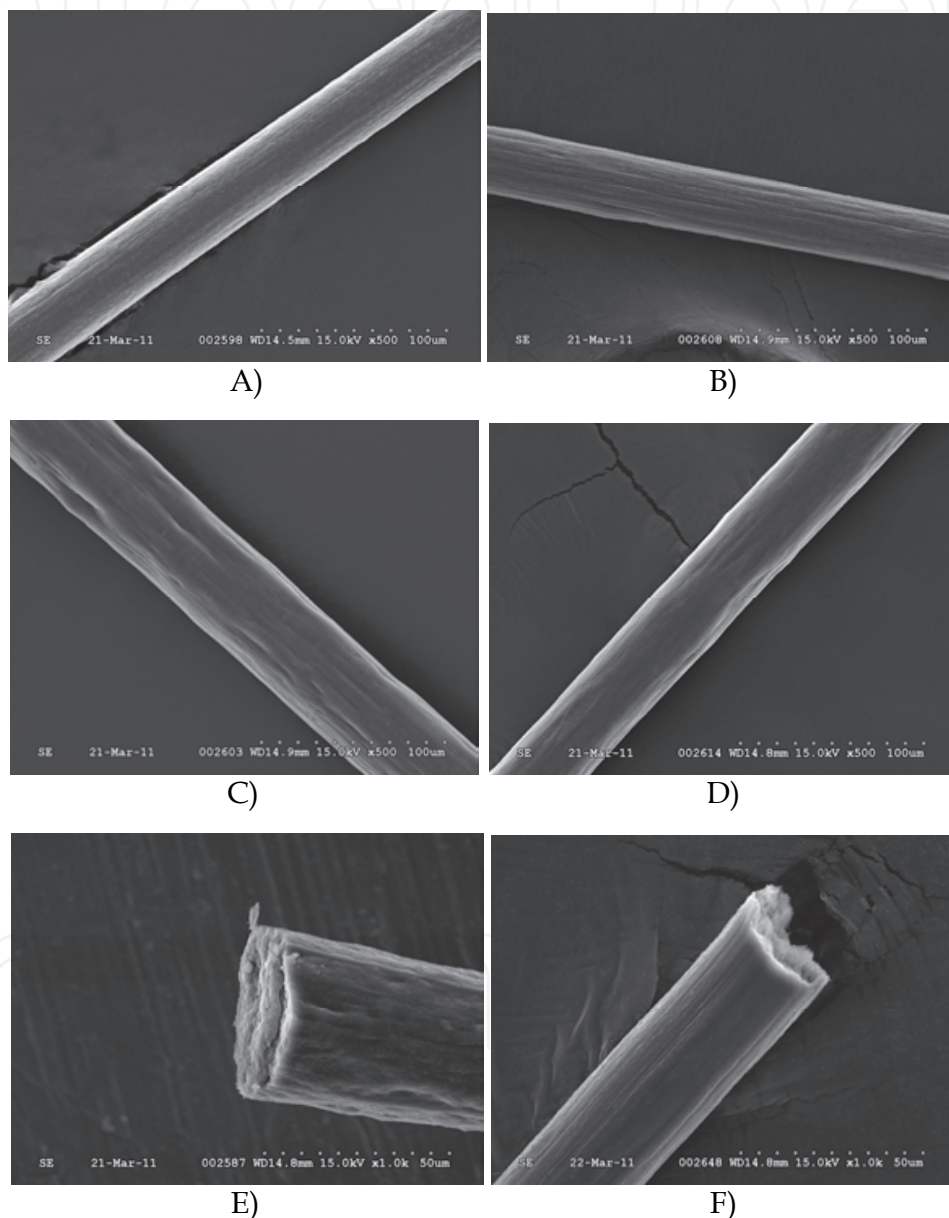


Fig. 5. SEM images of the surface of regenerated dragline silk fibers and blended fibers supplemented with recombinant MaSp1, MaSp2 or both spidroins. A) Regenerated dragline silk; B) Regenerated +1RCT_{MaSp1} (1:1); C) Regenerated + 1RCT_{MaSp2} (1:1); D) Regenerated +1RCT_{MaSp1} +1RCT_{MaSp2} (2:1:1). Fibroin mixture ratios are indicated in parentheses. All images were collected at a magnification of 500-1000X.

In addition to morphological differences, the fibers of varying formulations also exhibit markedly different mechanical behavior. When compared with the native dragline, the regenerated dragline silk has reduced modulus, extensibility, and toughness (Fig. 6). Supplementing the regenerated dragline silk with either recombinant 1RCT_{MaSp1} or 1RCT_{MaSp2} increases the elastic modulus, tensile strength, and toughness over the regenerated dragline silk alone. However, supplementing with both recombinant 1RCT_{MaSp1} and 1RCT_{MaSp2} appears to diminish the mechanical enhancements relative to either fibroin alone, suggesting that specific combinations of fibroins may provide optimal mechanical behavior. The molecular basis is currently being investigated. Taken together, our synthetic composite dragline silk fibers display higher tensile strength and extensibility relative to previous reports involving artificial fibers spun from truncated recombinant MaSp1 and MaSp2 lacking their C-termini (Brooks et al. 2008). This supports that the C-terminus contributes to enhancing the mechanical properties of the blended fibers.

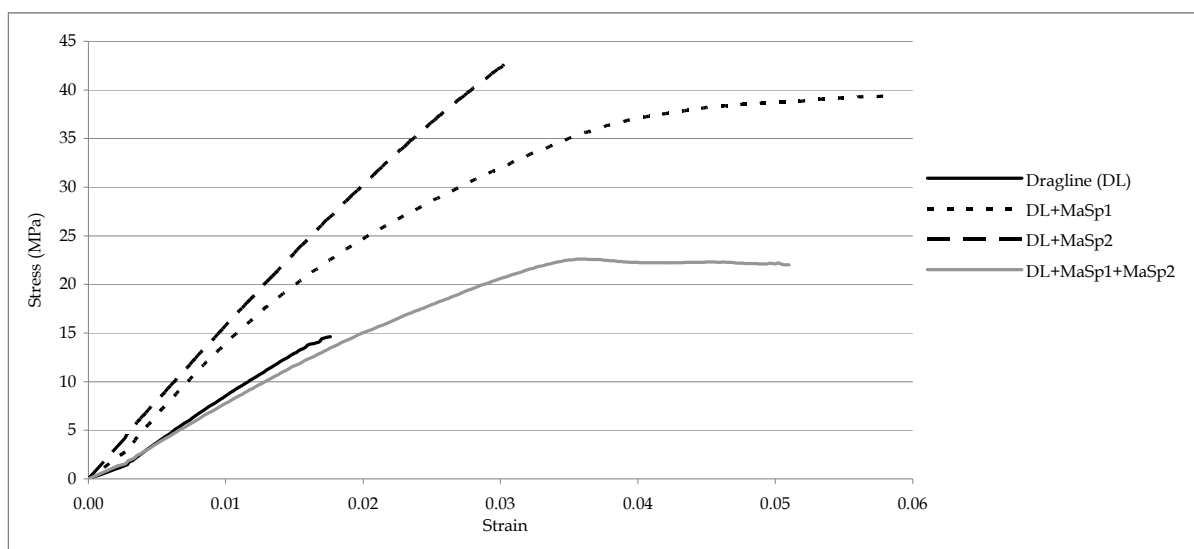
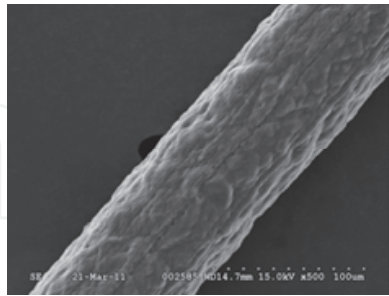


Fig. 6. Stress-strain curve performed using regenerated dragline and blended fibers containing MaSp1 and/or MaSp2. Plotted data are from a single fiber, but are representative of at least 4 different fibers.

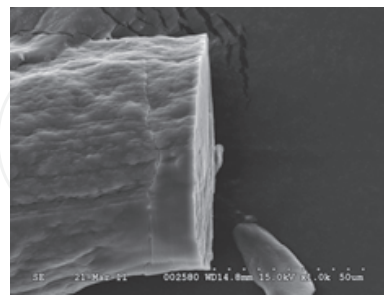
6.2.2 Composite silk fibers spun from regenerated aciniform and tubuliform silk proteins

Regenerated spider silk fibers spun from reconstituted egg sacs have not been reported in the scientific literature. Here we demonstrate that solubilization of black widow egg sacs with HFIP, followed by wet-spinning of the fibers into an isopropanol coagulation bath, can lead to a composite filament containing the fibroins AcSp1-like, TuSp1 and the ECP silk proteins. These artificial spider silk composites were spun from 20% (w/v) solutions of dissolved egg sacs. These are the first reported composite silk fibers that include native sized aciniform and tubuliform silk proteins blended together into a single filament. SEM studies demonstrated that the reconstituted egg case fibers contained diameter sizes approximately 90 μm (Fig. 7A). Analysis of the surface morphology of the fibers revealed more irregularities relative to the natural tubuliform silks (Compare Fig. 2D with 7A). The interior of fractured regenerated egg case fibers showed a solid core, suggesting that fibroin assembly was not impaired during the spinning process (Fig. 7B). Stress-strain curves performed on individual threads demonstrated

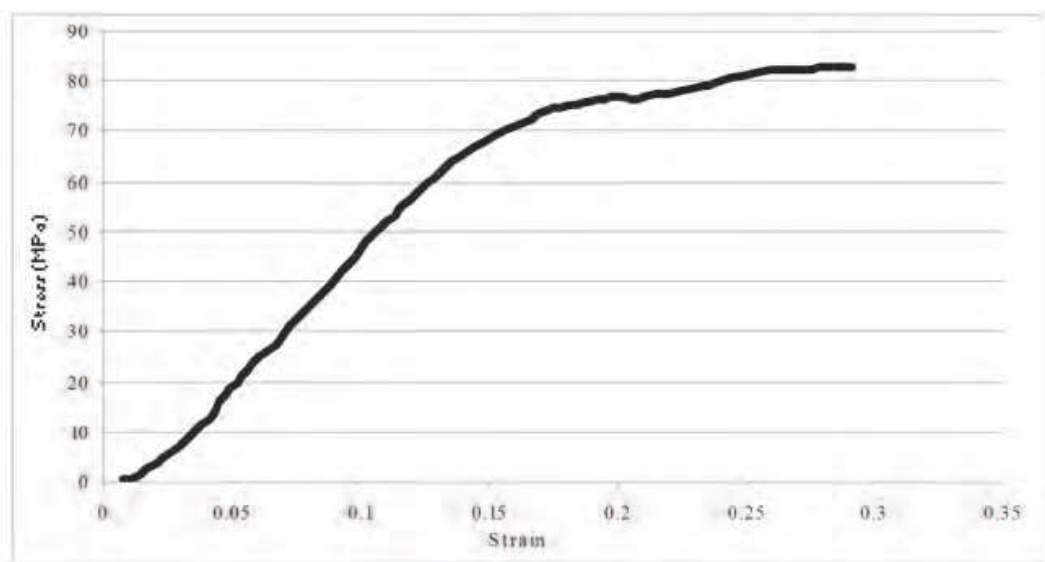
these composite fibers were 2-fold lower and an order of magnitude below the tensile strength of natural tubuliform silk (Fig. 7C). The % strain and tensile strength was 30% and 83 MPa, respectively. In the future, we plan on testing the biocompatibility of these fibers to determine their suitability for applications, such as tissue engineering.



A)



B)



C)

Fig. 7. Synthetic composite spider silk threads spun from regenerated egg case silk proteins. A-B) SEM analysis of regenerated and fractured egg case silk fibers, respectively; C) Stress-strain curve performed on regenerated egg case silk fibers.

7. Other applications

Methodologies to spin artificial silk fibers on a large-scale for industrial, medical, military and commercial use are progressing rapidly. In addition to spinning silk threads for a host of applications other uses for spider silk proteins are being pursued, including drug delivery and scaffolding materials. Both dragline silk fibers and egg sacs have been studied for their biocompatibility in a few human cell culture systems (Allmeling et al., 2006; Gellynck et al., 2008). Primary chondrocytes have been shown to attach and survive for several weeks on natural dragline silk and egg sacs (Gellynck et al., 2008). Natural dragline silk fibers have also been used to culture human primary Schwann cells and demonstrate these cells can

adhere and elongate (Allmeling et al., 2006). *In vivo* biocompatibility of native dragline silks has also been demonstrated by subcutaneous implantation in pigs (Vollrath et al., 2002), mice and rats (Gellynck et al., 2008). These studies have revealed that spider silk is well tolerated and generates little, if any, inflammatory response in the host animal.

Thus far, few *in vitro* studies have investigated the biocompatibility of recombinant spider silk fibroins. Because spidroin family members have different amino acid sequences within their block repeats, each member will require testing to determine whether certain family members are more suitable relative to the others. Advances in molecular biological techniques have facilitated the production of spider silk fibroins in sufficient quantities for the fabrication of various scaffolds. Three-dimensional scaffolds fabricated from recombinant dragline silk proteins have been shown to allow the attachment and migration of mouse fibroblast cells into the deep layers of the scaffolds within 7 days, implying that dragline silk fibers have excellent biocompatibility, stability and pore interconnectivity (Agapov et al., 2009). Additionally, recombinant spider silk proteins engineered with RGD cell-binding domains have been used successfully as biomaterial matrixes to enhance the differentiation of human bone marrow derived mesenchymal stem cells to bone-like tissue (Bini et al., 2006). Other uses for spider silk proteins include the production of recombinant spider silk particles as drug delivery vesicles (Lammel et al., 2011). Therefore, the use of spider silk fibroins for a host of different applications is currently being pursued in the scientific community.

8. Conclusion

This chapter highlights how regenerated dragline and tubuliform silk fibroins can be used to generate a spinning dope suitable for synthetic fiber production. It also explores the use of blending truncated recombinantly expressed dragline silk fibroins MaSp1 and MaSp2 with reconstituted dragline silk fibroins to generate composite spider silk fibers. With the identification of different spider silk cDNAs, coupled with the advancements in heterologous protein expression systems and the spinning process, the timing to explore the production of new engineering materials that integrate different mixtures of recombinant silk proteins and reconstituted silk proteins provides an exciting time for silk biology research. We have taken a novel approach, combining regenerated silk fibroins with recombinant proteins, to study the material properties of synthetic composite spider silk fibers. Our studies demonstrate that infusion of recombinant proteins into solubilized regenerated silk fibroin mixtures is a valid method to study fibroin function in spun composite silk fibers. It is also a method to generate a vast number of diverse synthetic composite fibers with a broad range of mechanical functions.

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10. References

- Agapov, I., Pustovalova, O. L., Moisenovich, M. M., Bogush, V. G., Sokolova, O. S., Sevastyanov, V. I., et al. (2009). Three-dimensional scaffold made from recombinant spider silk protein for tissue engineering. *Dokl Biochem Biophys*, 426, 127-130.
- Allmeling, C., Jokuszies, A., Reimers, K., Kall, S., & Vogt, P. M. (2006). Use of spider silk fibres as an innovative material in a biocompatible artificial nerve conduit. *J Cell Mol Med*, 10(3), 770-777.
- Ando, Y., Okano, R., Nishida, K., Miyata, S., & Fukade, E. (1980). Piezoelectric and related properties of hydrated silk fibroin. *Rep Prog Polymer Physics Japan*, 23, 775-778.
- Askarieh, G., Hedhammar, M., Nordling, K., Saenz, A., Casals, C., Rising, A., Johansson, J., & Knight, S. (2010). Self-assembly of spider silk proteins is controlled by a pH-sensitive relay. *Nature*, 465(7295), 236-238.
- Ayoub, N. A., Garb, J. E., Tinghitella, R. M., Collin, M. A., & Hayashi, C. Y. (2007). Blueprint for a high-performance biomaterial: full-length spider dragline silk genes. *PLoS ONE*, 2, e514.
- Bini, E., Foo, C. W., Huang, J., Karageorgiou, V., Kitchel, B., & Kaplan, D. L. (2006). RGD-functionalized bioengineered spider dragline silk biomaterial. *Biomacromolecules*, 7(11), 3139-3145.
- Blasingame, E., Tuton-Blasingame, T., Larkin, L., Falick, A. M., Zhao, L., Fong, J., Vaidyanathan, V., Visperas, A., Geurts, P., Xu, X., La Mattina, C., & Vierra, C. (2009). Pyriform spidroin 1, a novel member of the silk gene family that anchors dragline silk fibers in attachment discs of the black widow spider, *Latrodectus hesperus*. *J Biol Chem*, 284(42), 29097-29108.
- Brooks, A. E., Nelson, S. R., Jones, J. A., Koenig, C., Hinman, M., Stricker, S., & Lewis, R. (2008). Distinct contributions of model MaSp1 and MaSp2 like peptides to the mechanical properties of synthetic major ampullate silk fibers as revealed in silico. *Nanotechnol Sci Appl*, 1, 9-16.
- Casem, M. L., Turner, D., & Houchin, K. (1999). Protein and amino acid composition of silks from the cob weaver, *Latrodectus hesperus* (black widow). *Int. J. Biol. Macromol.*, 24(2-3), 103-108.
- Casem, M. L., Tran, L. P., & Moore, A. M. (2002). Ultrastructure of the major ampullate gland of the black widow spider, *Latrodectus hesperus*. *Tissue Cell*, 34(6), 427-436.
- Chen, X., Knight, D. P., & Vollrath, F. (2002). Rheological characterization of *Nephila* spidroin solution. *Biomacromolecules*, 3(4), 644-648.
- Craig, C. L., Riekel, C., Herberstein, M. E., Weber, R. S., Kaplan, D., & Pierce, N. E. (2000). Evidence for diet effects on the composition of silk proteins produced by spiders. *Mol. Biol. Evol.*, 17(12), 1904-1913.
- Dicko, C., Vollrath, F., & Kenney, J. M. (2004). Spider silk protein refolding is controlled by changing pH. *Biomacromolecules*, 5(3), 704-710.
- Du, N., Liu, X. Y., Narayanan, J., Li, L., Lim, M. L., & Li, D. (2006). Design of superior spider silk: from nanostructure to mechanical properties. *Biophys J*, 91(12), 4528-4535.
- Fahnestock, S. R., & Bedzyk, L. A. (1997). Production of synthetic spider dragline silk protein in *Pichia pastoris*. *Appl. Microbiol. Biotechnol.*, 47(1), 33-39.
- Foelix, R. (1996). *Biology of spiders*. New York: Oxford University Press.
- Gellynck, K., Verdonk, P. C., Van Nimmen, E., Almqvist, K. F., Gheysens, T., Schoukens, G., Van Langenhove, L., Kiekens, P., Mertens, J., & Verbruggen, G. (2008). Silkworm

- and spider silk scaffolds for chondrocyte support. *J Mater Sci Mater Med*, 19(11), 3399-3409.
- Gosline, J. M., DeMont, M. E., Denny, M. W. (1986). The structure and properties of spider silk. *Endeavour*, 10, 31-43.
- Gosline, J.M., Guerette, P.A., Ortlepp, C.S., & Savage, K.N. (1999). The mechanical design of spider silks: from fibroin sequence to mechanical function. *J. Exp. Biol.*, 202(Pt 23), 3295-3303.
- Geurts, P., Zhao, L., Hsia, Y., Gnesa, E., Tang, S., Jeffery, F., La Mattina, C., Franz, A., Larkin, L., & Vierra, C. Synthetic spider silk fibers spun from Pyriform Spidroin 2, a glue silk protein discovered in orb-weaving spider attachment discs. *Biomacromolecules*, 11(12), 3495-3503.
- Guerette, P. A., Ginzinger, D. G., Weber, B. H., & Gosline, J. M. (1996). Silk properties determined by gland-specific expression of a spider fibroin gene family. *Science*, 272(5258), 112-115.
- Hagn, F., Eisoldt, L., Hardy, J. G., Vendrely, C., Coles, M., Scheibel, T., & Kessler, H. (2010). A conserved spider silk domain acts as a molecular switch that controls fibre assembly. *Nature*, 465(7295), 239-242.
- Hayashi, C. Y., Blackledge, T. A., & Lewis, R. V. (2004). Molecular and mechanical characterization of aciniform silk: uniformity of iterated sequence modules in a novel member of the spider silk fibroin gene family. *Mol. Biol. Evol.*, 21(10), 1950-1959.
- Hedhammar, M., Bramfeldt, H., Baris, T., Widhe, M., Askarieh, G., Nordling, K., Aulock, S., & Johansson, J. (2010). Sterilized recombinant spider silk fibers of low pyrogenicity. *Biomacromolecules*, 11(4), 953-959.
- Hinman, M. B., & Lewis, R. V. (1992). Isolation of a clone encoding a second dragline silk fibroin. *Nephila clavipes* dragline silk is a two-protein fiber. *J. Biol. Chem.*, 267(27), 19320-19324.
- Hu, X., Lawrence, B., Kohler, K., Falick, A. M., Moore, A. M., McMullen, E., Jones, P., & Vierra, C. (2005). Araneoid egg case silk: a fibroin with novel ensemble repeat units from the black widow spider, *Latrodectus hesperus*. *Biochemistry*, 44(30), 10020-10027.
- Hu, X., Vasanthavada, K., Kohler, K., McNary, S., Moore, A. M., & Vierra, C. A. (2006a). Molecular mechanisms of spider silk. *Cell Mol Life Sci*, 63(17), 1986-1999.
- Hu, X., Kohler, K., Falick, A. M., Moore, A. M., Jones, P. R., & Vierra, C. (2006b). Spider egg case core fibers: trimeric complexes assembled from TuSp1, ECP-1, and ECP-2. *Biochemistry*, 45(11), 3506-3516.
- Hu, X., Yuan, J., Wang, X., Vasanthavada, K., Falick, A. M., Jones, P. R., La Mattina, C. & Vierra, C. (2007). Analysis of aqueous glue coating proteins on the silk fibers of the cob weaver, *Latrodectus hesperus*. *Biochemistry*, 46(11), 3294-3303.
- Huemmerich, D., Helsen, C. W., Quedzuweit, S., Oschmann, J., Rudolph, R., & Scheibel, T. (2004). Primary structure elements of spider dragline silks and their contribution to protein solubility. *Biochemistry*, 43(42), 13604-13612.
- Jeffery, F., La Mattina, C., Tuton-Blasingame, T., Hsia, Y., Gnesa, E., Zhao, L., Franz, A., & Vierra, C. (2011) Microdissection of black widow spider silk-producing glands. *J Vis Exp*(47).
- Knight, D. P., Knight, M. M., & Vollrath, F. (2000). Beta transition and stress-induced phase separation in the spinning of spider dragline silk. *Int. J. Biol. Macromol.*, 27(3), 205-210.

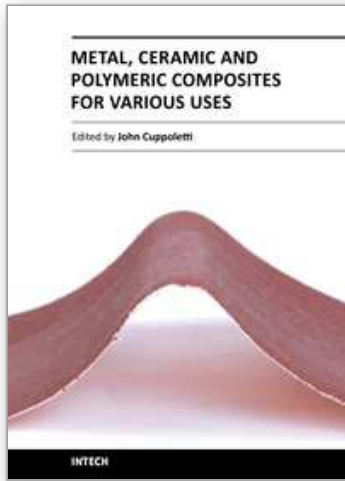
- Knight, D. P., & Vollrath, F. (2001). Changes in element composition along the spinning duct in a *Nephila* spider. *Naturwissenschaften*, 88(4), 179-182.
- Lammel, A., Schwab, M., Hofer, M., Winter, G., & Scheibel, T. (2011). Recombinant spider silk particles as drug delivery vehicles. *Biomaterials*, 32(8), 2233-2240.
- Lawrence, B.A., Vierra, C.A., & Moore, A.M. (2004). Molecular and mechanical properties of major ampullate silk of the black widow spider, *Latrodectus hesperus*. *Biomacromolecules*, 5(3), 689-695.
- Lazaris, A., Arcidiacono, S., Huang, Y., Zhou, J. F., Duguay, F., Chretien, N., Welsch, E., Soares, J., & Karatzas, C. (2002). Spider silk fibers spun from soluble recombinant silk produced in mammalian cells. *Science*, 295(5554), 472-476.
- Lefevre, T., Boudreault, S., Cloutier, C., & Pezolet, M. (2008). Conformational and orientational transformation of silk proteins in the major ampullate gland of *Nephila clavipes* spiders. *Biomacromolecules*, 9(9), 2399-2407.
- Lefevre, T., Boudreault, S., Cloutier, C., & Pezolet, M. (2011). Diversity of molecular transformations involved in the formation of spider silks. *J Mol Biol*, 405(1), 238-253.
- Lewis, R. V. (2006). Spider silk: ancient ideas for new biomaterials. *Chem Rev*, 106(9), 3762-3774.
- Lin, L. H., Edmonds, D. T., & Vollrath, F. (1995). Structural engineering of an orb-spider's web. *Nature*, 373, 146-148.
- Lin, Z., Huang, W., Zhang, J., Fan, J. S., & Yang, D. (2009). Solution structure of eggcase silk protein and its implications for silk fiber formation. *Proc Natl Acad Sci U S A*, 106(22), 8906-8911.
- Liivak, O., Flores, A., Lewis, L., & Jelinski, L. W. (1997). Conformation of the polyalanine repeats in minor ampullate gland silk of the spider *Nephila clavipes*. *Macromolecules*, 30, 7127-7130.
- Lombardi, S., & Kaplan, D. L. (1990). The Amino Acid Composition of Major Ampullate Gland Silk (Dragline) of *Nephila clavipes* (Araneae, Tetragnathidae). *J. Arachnol.*, 18, 297-306.
- Michal, C. A., Simmons, A. H., Chew, B. G., Zax, D. B., & Jelinski, L. W. (1996). Presence of phosphorus in *Nephila clavipes* dragline silk. *Biophys J*, 70(1), 489-493.
- Nova, A., Keten, S., Pugno, N. M., Redaelli, A., & Buehler, M. J. (2010). Molecular and nanostructural mechanisms of deformation, strength and toughness of spider silk fibrils. *Nano Lett*, 10(7), 2626-2634.
- Ortiz, R., Cespedes, W., Nieves, L., Robles, I. V., Plazaola, A., File, S., et al. (2000). Small ampullate glands of *Nephila clavipes*. *J Exp Zool*, 286(2), 114-119.
- Pouchkina, N. N., Stanchev, B. S., & McQueen-Mason, S. J. (2003). From EST sequence to spider silk spinning: identification and molecular characterisation of *Nephila senegalensis* major ampullate gland peroxidase NsPox. *Insect Biochem Mol Biol*, 33(2), 229-238.
- Scheller, J., Guhrs, K. H., Grosse, F., & Conrad, U. (2001). Production of spider silk proteins in tobacco and potato. *Nat. Biotechnol.*, 19(6), 573-577.
- Schmidt, M., Romer, L., Strehle, M., & Scheibel, T. (2007). Conquering isoleucine auxotrophy of *Escherichia coli* BLR(DE3) to recombinantly produce spider silk proteins in minimal media. *Biotechnol Lett*, 29(11), 1741-1744.
- Shao, Z., Vollrath, F., Yang, Y., & Thogersen, H. (2003). Structure and Behavior of Regenerated Spider Silk. *Macromolecules*, 36, 1157-1161.

- Simmons, A. H., Michal, C. A., & Jelinski, L. W. (1996). Molecular orientation and two-component nature of the crystalline fraction of spider dragline silk. *Science*, 271(5245), 84-87.
- Sponner, A., Unger, E., Grosse, F., & Weisshart, K. (2005). Differential polymerization of the two main protein components of dragline silk during fibre spinning. *Nat. Mater.*, 4(10), 772-775.
- Sponner, A., Vater, W., Monajembashi, S., Unger, E., Grosse, F., & Weisshart, K. (2007). Composition and hierarchical organisation of a spider silk. *PLoS ONE*, 2(10), e998.
- Stark, M., Grip, S., Rising, A., Hedhammar, M., Engstrom, W., Hjalms, G., et al. (2007). Macroscopic fibers self-assembled from recombinant miniature spider silk proteins. *Biomacromolecules*, 8(5), 1695-1701.
- Szela, S., Avtges, P., Valluzzi, R., Winkler, S., Wilson, D., Kirschner, D., & Kaplan, D. (2000). Reduction-oxidation control of beta-sheet assembly in genetically engineered silk. *Biomacromolecules*, 1(4), 534-542.
- Teule, F., Cooper, A. R., Furin, W. A., Bittencourt, D., Rech, E. L., Brooks, A., & Lewis, R. (2009). A protocol for the production of recombinant spider silk-like proteins for artificial fiber spinning. *Nat Protoc*, 4(3), 341-355.
- Tso, I. M., Wu, H. C., & Hwang, I. R. (2005). Giant wood spider *Nephila pilipes* alters silk protein in response to prey variation. *J. Exp. Biol.*, 208(Pt 6), 1053-1061.
- Vasanthavada, K., Hu, X., Falick, A. M., La Mattina, C., Moore, A. M., Jones, P. R., Yee, R., Reza, R., Tuton, T., & Vierra, C. (2007). Aciniform spidroin, a constituent of egg case sacs and wrapping silk fibers from the black widow spider *Latrodectus hesperus*. *J Biol Chem*, 282(48), 35088-35097.
- Vollrath, F., Wen Hu, X., & Knight, D. P. (1998). Silk production in a spider involves acid bath treatment. *Proc. R. Soc. B*, 263, 817-820.
- Vollrath, F., Barth, P., Basedow, A., Engstrom, W., & List, H. (2002). Local tolerance to spider silks and protein polymers in vivo. *In Vivo*, 16(4), 229-234.
- Winkler, S., Szela, S., Avtges, P., Valluzzi, R., Kirschner, D. A., & Kaplan, D. (1999). Designing recombinant spider silk proteins to control assembly. *Int J Biol Macromol*, 24(2-3), 265-270.
- Winkler, S., Wilson, D., & Kaplan, D. L. (2000). Controlling beta-sheet assembly in genetically engineered silk by enzymatic phosphorylation/dephosphorylation. *Biochemistry*, 39(41), 12739-12746.
- Xia, X. X., Qian, Z. G., Ki, C. S., Park, Y. H., Kaplan, D. L., & Lee, S. Y. (2010). Native-sized recombinant spider silk protein produced in metabolically engineered *Escherichia coli* results in a strong fiber. *Proc Natl Acad Sci U S A*, 107(32), 14059-14063.
- Xu, M., & Lewis, R. V. (1990). Structure of a protein superfiber: Spider Dragline Silk. *Proc. Natl. Acad. Sci.*, 87, 7120-7124.
- Xu, H. T., Fan, B. L., Yu, S. Y., Huang, Y. H., Zhao, Z. H., Lian, Z. X., Dai, Y.P., Wang, L.L., Liu, Z.L., Fei, J., & Li, N. (2007). Construct synthetic gene encoding artificial spider dragline silk protein and its expression in milk of transgenic mice. *Anim Biotechnol*, 18(1), 1-12.
- Yang, J., Barr, L. A., Fahnestock, S. R., & Liu, Z. B. (2005). High yield recombinant silk-like protein production in transgenic plants through protein targeting. *Transgenic Res.*, 14(3), 313-324.

- Zhao, A., Zhao, T., Sima, Y., Zhang, Y., Nakagaki, K., Miao, Y., et al. (2005). Unique molecular architecture of egg case silk protein in a spider, *Nephila clavata*. *J. Biochem. (Tokyo)*, 138(5), 593-604.
- Zhao, A. C., Zhao, T. F., Nakagaki, K., Zhang, Y. S., Sima, Y. H., Miao, Y. G., Kajiura, Z., Nagata, Y., Takadera, M., & Nakagaki, M. (2006). Novel molecular and mechanical properties of egg case silk from wasp spider, *Argiope bruennichi*. *Biochemistry*, 45(10), 3348-3356.

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