



Spider Silk - Structure, Properties and Spinning

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ABSTRACT

Many of the natural fibers offer excellent properties suitable for various applications in apparel and non-apparel areas. Spider silk is a filamentous natural protein fiber produced by the spiders. Dragline silk produced by the spider offers superior properties than many of the natural and synthetic fibers. The natural spinning process, chemical composition, structure and properties of spider silk had remained mystery for a long time. Systematic attempts made in the biological aspects, structure of the silk proteins have become fruitful in spinning and regenerating this wonder fiber.

Keywords: Dragline, Major Ampullate, Nephila, Interphase, Beta sheet

INTRODUCTION

The term silk normally refers to a wide range of continuous filaments spun by the several species of Lepidoptera and Arthropoda, used for building structures for various purposes including prey capture. Silk filaments spun by spiders and silkworms are found to possess superior properties than other silk producing insects and more than 2500 orb weaving species exist worldwide [1 - 4]. Spiders have six or seven sets of glands, each producing different fiber and these glands remained undifferentiated, early in the evolution [4 - 7]. The spinnerets, microscopic tubes originating from glands, are classified into major, minor ampullate and the term "ampulla" is used to describe the distal part of the secretary zone [8]. Unlike synthetic polymers, the biopolymers are composed of numerous monomers arranged in a strictly

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controlled manner [9]. Many attempts have been made continuously in the past to harvest and convert spider silk filaments into fabric form [4, 10, 11].

SPIDER WEB AND TYPES OF SPIDER SILK

Prior to the exploration of the structure and properties of spider silks, construction and design of webs have been the major area of focus [3, 4, 12 - 34]. The spider webs can take a variety of forms but the most common type is the orb web. Different families of spiders like *Araneus*, *Nephila* builds orb-web and other families of spiders construct tangle and sheet webs [33, 35]. Orb-web spiders invest little energy in searching the prey and majority in silk synthesis and construction of the webs. Fig. 1 shows various threads and the web constructed by the orb-weaving spiders- *Araneus* and *Nephila*.

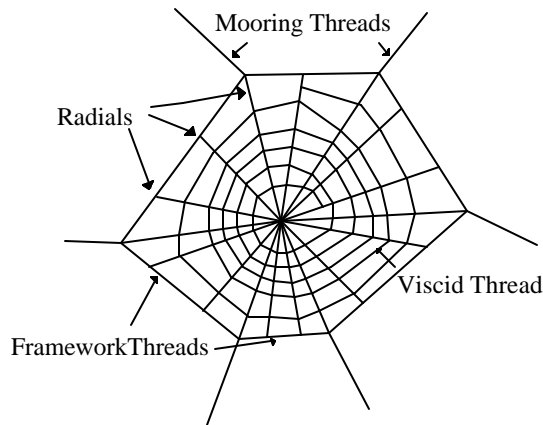


Fig. 1 Schematic Diagram of Spider Web

An orb web has several spokes laid outward from a common origin, which varies with the species of spiders [31]. The orb webs are often constructed with an orientation to avoid web damage due to the air drag caused by prey capture [24]. In a three dimensional web, the energy required to stop a moving insect is dissipated mainly by breaking some of the strands while in a two dimensional orb web, it is achieved through stretching the spiral threads [29]. Due to high-energy requirement in protein synthesis, only the damaged parts of the web are reconstructed instead of complete web and large portions of the web are repaired through the enzyme digestion and recycling [28, 34]. The design of orb webs varies among the species, individuals, and day-to-day for an individual and also within the web. Webs of young spiders are more circular than that of adults and hungry spiders construct smaller webs. Based on the vibrations of the strands, the spider locates the prey accurately. In a web, placing of capture threads in the upper half region becomes difficult since it necessitates lifting of abdomen and the spinnerets above the head and so the lower web region is of greater prey capture value and the spider increases this section of the web and decreases the extent of the upper web region [27]. This asymmetry varies with species, age, weight of the spiders and web size but upper half is essential to maintain the functional and stable webs.

The orb-weaving spiders are able to synthesize as many as seven different types

of silk [13-15] including dragline by drawing liquid crystalline proteins from separate gland-spinneret complex. The various types of silk produced by a spider along with their functions are listed in the Table 1. Dragline, minor ampullate and viscid silks form the major portions of the orb web and the dragline silk, in the form of mooring threads, framework and pre-tensioned radial threads, dominates the web structure [24]. Multi strands of spider silks are laid in the web to withstand adverse conditions and impact created by fast moving prey. The perfume-coated dragline helps to find their mates, swing from place to place, store the food and egg and for reproduction also [19]. Minor ampullate silk produced by median spinneret is characterized by higher elasticity and low strength [26]. Minor ampullate silks are generally finer than major ampullate silk with better uniformity but their properties are highly uncertain [21]. Capture threads with glue droplets ranging from 7 – 29 micron are used for the aerodynamic damping of impact caused by the flying insects [24, 36]. Capture threads produced by the flagelliform glands of *Nephila Clavipes* are highly compliant. Both *Araneus* and *Nephila* coat their capture threads with an aqueous solution that forms sticky droplets, which enhance the damping and harvest water from the air [25]. Its principal function is to absorb and dissipate the kinetic energy of captured flying insects [3]. The water content of the coated substance plasticize the core threads and

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renders them more elastic to maintain overall toughness ever after contraction through kinetically free molecular chains. The capture silk of *Araneus* consists of a pair of fibers coated with composition that contains 80% water, and amino acids, glycoprotein, lipids, salts and low molecular weight compounds to the extent of 20 – 30%, form regularly spaced droplets along the filament [30]. The chemical composition of the aqueous solution of the adhesive spiral varies among the individuals both qualitatively and quantitatively mainly

due to physical environment, diet, web recycling, and onto genetic changes in the web chemistry. While stretching, the core filament inside the droplet extends to a level of 200 %, which prevents the rebound of the prey after catching [25, 37 - 39]. When this silk fibroin is treated to remove the water; it also exhibits properties that are similar to major ampullate silk [22]. Capture silk contains less than 5% crystallites by volume. It has a low initial modulus 3 MPa and high extensibility [14].

Table 1 Type of Glands, Silks and Their Functions

Silk	Gland	Spinneret Used	Function	Amino acid composition
Dragline	Major Ampullate	Anterior/ Median	Orb web frame, radii	Glycine(37%), Alanine(18%) small chains (62%), polar (26%)
Viscid	Flagelliform	Posterior	Prey capture, sticky spiral	Glycine (44%), Proline (21%), small side chains (56%), polar (17%)
Glue-like	Aggregate	Posterior	Prey capture, attachment	Glycine (14%), Proline (11%), Polar glue (49%), small side chains (27%)
Minor	Minor Ampullate	Anterior/ Median	Orb web frame	Glycine (43%), Alanine (37%), small side chains (85%), polar (26%)
Cocoon	Cylindrical	Median/ Posterior	Reproduction	Serine (28%), Alanine (24%), small side chains (61%), polar (50%)
Wrapping	Aciniform	Median / Posterior	Wrapping captured prey	Serine (15%), Glycine (13%), Alanine (11%), small side chains (40%), polar (47%)
Attachment	Piriform	Anterior	Attachment	Serine (15%), Sidechains(32%), Polar (58%)

SPINNING OF SPIDER SILK

Spiders and Natural Spinning Process

Many spiders are active at night and their colorations are usually orange, brown, grey and black, to reduce the spiders' visibility during day time. *Nephila clavipes* spider is about 2 ½" in size, orange and black in color with a weight of 90 - 1456 mg [33, 40, 41, 42]. Silk secreting systems of spiders and insects are homologous and linked to the cursal gland and cuticular secretions [6]. Systematic studies of natural spinning process of spider silk have shown a marvelous process of filament making from the delicate glands at very low temperature, using water as solvents [14, 22, 23, 28, 29, 31, 32, 34, 43 - 50].

Cephalothoraxes of the spider attached to an unsegmented abdomen, which has spinnerets at the posterior end [33]. *N. clavipes* spider has three pairs of spinnerets namely, anterior lateral, posterior lateral and posterior median. The largest major ampullate gland secretes dragline silk protein, exits from the anterior lateral spinneret (Fig. 2). Secretions of proximal region and the distal region together form spider silk. Proximal region secretions are rich in tyrosine residues, sulfhydryl linkages and acidophilic nature. They form core of the silk while secretions of distal zone form coating of the fiber, which lacks tyrosine, sulfur contents. The duct, connecting the spinneret and the distal zone, causes the orientation of protein molecules as well as

water removal. Final coating consisting of four layers is given to the threads in the third limb of the 'S' shaped duct, close to the start of the drawn down taper [8]. The duct has a thin cuticle which acts as a dialysis membrane, removes water and sodium ions out of the lumen and adds potassium,

possibly surfactants, lubricants and phase separation promoters into the lumen [48]. A liquid layer is given in the final limb through an acidic bath. The valve present just prior to the spinneret controls breaking and restarting of the spinning process [8].

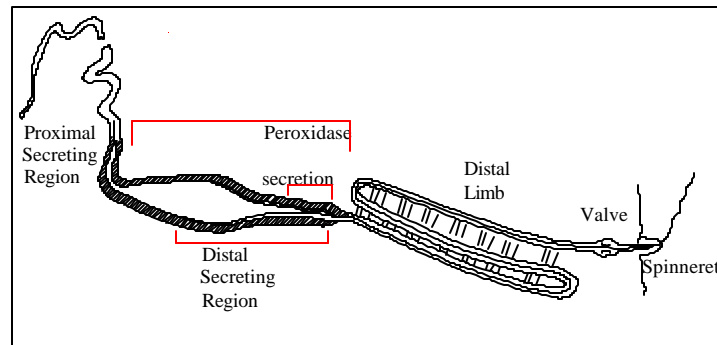


Fig. 2 Natural Spinning System of Dragline Silk

The hyperbolic shape of the spinning duct imparts stress and orientation to the dope during extrusion, leads to stress-induced crystallization of the material and the friction with the wall of the spider's spinning duct provides force needed to trigger the change in conformation. Proton pumps secrete hydrogen ions to render the solution more acidic near the exit from the duct and the resulting gradients in pH, polymer concentration and potassium concentration are believed to contribute to the structural transitions. The extension of molecules under stress enables them to join together with hydrogen bonds, like zip fasteners and give anti parallel β conformation in the final thread. As silk protein molecules aggregate and crystallize, the hydrophilic groups are hidden into the interior and transformed into hydrophobic surface that facilitates the loss of water from the surface of the extruded thread [45, 51]. The water removed from the dope is reabsorbed by the silk to avoid excessive water loss [48].

PROCESS CONDITIONS AND THEIR INFLUENCES

A mature *Nephila* produces dragline silk fiber at approximately 1 cm/sec during web construction and can increase up to 10 times faster during a rapid descent [52]. Spider silk spun under water displays greater stiffness and resilience compared to silk spun naturally into air [53]. The diameter of the silk can be controlled by the valve located at the end of the duct [47]. The spiders have the ability to withstand the temperature variation of up to 30°C and humidity variation of up to 70% [20]. The spiders can easily modify the spinning conditions by moving speed, building the webs in different times in a day, i.e. at different temperatures. Spinning speed has less influence on the diameter of the filament compared to the temperature but strong influence on the toughness. As the feed stock flows, the droplets coalesce and progressively stretched out by elongational flow to form silk filaments with elongated canaliculi.

Variability in the silk spun by the spider exists at different levels like inter-specific (between species), intra-specific (within same species) and intra individual levels [54]. Differences exist in the composition and properties at inter-specific level and properties of the silk in the case of the intra specific level. The factors that affect variations in silk structure and properties include body dimensions, body weight, rate and temperature of reeling, spinning direction [17, 25]. Composition of silks produced by herbivorous spiders is rich in Glycine, Alanine and Serine and can be predicted to a larger extent. However, the silk produced by predatory spider, cannot be predicted due to the different types of prey [40, 55]. Dietary compositions of herbivorous spiders are energy rich and poor in protein content whereas the diet of predatory spiders is more diverse and rich in protein. Competition for limited or fluctuating supplies of amino acid perhaps has resulted in the evolution of two different kinds of glands to secrete protein glues and silk fibroin. The spider produces the thread on a very strict energy budget using liquid crystalline polymer.

Samples collected from the undisturbed climbing of *Argiope* spider reveal [41] the consistent intrinsic tensile properties and independent of the weight of the spider, age of spider, relaxation of the spinneret dimensions and the speed, which the vertical spinning, the spider needs to be protected from falling, regardless of the weight of the spider and this can be achieved by increased diameter of the filament. High level of spidroin and metabolic activity is not required in the horizontal spinning due to lower level of risk associated in these directions.

Rheology of Spider Silk Protein

Solutions of silk protein show the tendency to gel even at rest and the liquid crystalline dope differs from isotropic solutions [2, 48, 56 - 60]. The silk dope consists of a concentrated protein, over 30 - 40% [3, 50]. Dope extracted from the MA gland shows a viscosity of about 3500 Pa. Sec. [61]. While

liquid crystalline viscosity depends on shear rate, stress along and across the flow lines of the liquid, it is highly dependent on the concentration of the solution. By controlling solvent saturation, i.e. water content of liquid crystalline spinning solution, the spider can reduce the metabolic cost of silk production. The protein droplets obtained from the major ampullate glands of *Nephila clavipes* shows alkalinity and optical isotropicity indicating the absence of any ordered structures but exhibits nematic structure on loss of water. The pressure required to push the dope is reduced due by shear induced transition to a liquid crystalline phase, localized slip of polymer solution on the wall or lubrication / watery layer surrounding the fibroin. The elongation flow results in the strain hardening and due to chain stretching of entangled spidroin macromolecules inhibit the capillary breaks of spun threads are limited. Protein-protein interactions due to hydrophobic effects between non polar amino acids, dispersion forces and finally the electrostatic attraction between charge bearing arginine residues contribute to the effective binding potential between fibroin molecules [50, 62].

As the LCP feedstock passes down, loss of water occurs, a nematic structure is formed along with the molecular orientation. The structure follows a nematic discotic type and secondary structure from helix to sheet forms of the crystal structure. This transformation to liquid crystalline phase makes solutions of silk proteins spinnable despite their high concentration in the gland. The dilute solutions of the spider silk exhibit a shear thinning behavior with increasing shear rate and the concentrated solutions exhibit a shear thinning and shear thickening behavior. Very low critical shear rates are required to produce shear thickening behavior with the increasing concentration at pH 6.3. Interestingly, this helps the spiders to construct the webs at a low shear rate and high concentration levels. Addition of potassium ions to the dope solution in the distal limb and application of shear inside the duct result in the phase separation, formation of nanofibrils and aggregation

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into insoluble form. Liquid crystalline behavior of spider silk helps in many ways for the production of filaments, like, it is virtually free from the uncontrolled reorientation of molecules, the force required to spin a thread is less, and alignment of the molecules in the dope reduces the formation of defects.

STRUCTURE AND PROPERTIES

Spider silk has drawn attention from all the sections of engineering on account of its superior properties compared to existing fibrous materials. Chemical, physical properties and their relationship of silkworm silk, spider silk have been reviewed [63 - 66]. The structural features of dragline silks are not altered even after prolonged storage.

Chemical Composition

Chemical composition in terms of fibroin molecule, amino acid sequences, amino acid compositions, amino acid residues and various end groups have been studied elaborately [3, 13, 16, 26, 37, 47, 50, 67 - 76]. Molecular weight (Mn) of dragline silk protein of *Nephila clavipes* is 720, 000 and 96 000 to 520 000 for proteins obtained from glands and spun fibers [3, 13]. Major ampullate silk fibroin of dragline spider contains multiple repeats of long polyalanine block and glycine / proline rich blocks [14, 77, 78]. About 5 – 10 residues of polyalanine form a beta sheet and account for most of the crystalline fraction. Dragline silk can be considered as the block copolymer with polyalanine blocks and of glycine rich domains that imparts stiffness, strength and toughness [68].

Amino Acid	Sericin	Fibroin	Wool Keratin	Spider Silk
Glycine	13.9	43.7	8.4	37.1
Alanine	5.9	28.8	5.5	21.1
Valine	2.7	2.2	5.6	1.8
Leucine	1.1	0.5	7.8	3.8
Isoleucine	0.7	0.7	3.3	0.9
Serine	33.4	11.9	11.6	4.5
Theronine	9.7	0.9	6.9	1.7
Aspartic Acid	16.7	1.3	5.9	2.5
Glutamic Acid	4.4	1.0	11.3	9.2
Phenylalanine	0.5	0.6	2.8	0.7
Tyrosine	2.6	5.1	3.5	---
Lysine	3.3	0.3	2.6	0.5
Histidine	1.3	0.2	0.9	0.5
Arginine	3.1	0.5	6.4	7.6
Proline	0.6	0.5	6.8	4.3
Tryptophan	0.2	0.3	0.5	2.9
Cystine	0.1	0.2	9.8	0.3
Methionine	0.04	0.1	0.4	0.4

Table 2 Amino Acid Composition (mole %) of Spider dragline silk and Other Protein Fibers

The chemical compositions of various silks vary with the type of function they need to perform and fibroin composed of twenty odd alpha amino acids [13, 67]. Fibroin content of the *Nephila* has been found to be about 90% at 65% RH [79]. All types of silk share four types of amino acid motifs comprising of GPGGX/GPGQQ, GGX, Poly (Ala) or Poly (Gly-Ala) and spacer sequences to

prevent premature fiber formation within glands, align various structural regions within the fiber and to provide additional surfaces for critical interactions. GPGXX pentapeptide repeat conforms to a spiral and the proline residue behaves as the focal point for the retraction energy after stretching. The small side chains in the polymer consist of glycine, alanine and

serine and the polar substance consists of aspartic acid, threonine, serine, glutamic acid, tyrosine, lysine, histidine and arginine. The various amino acid contents are found to be Alanine – 29%, Glycine – 40%, Proline – 3%, Tyrosine – 4%, Glutamine 10% [47, 67, 68]. Hydrogen bonds between carbonyl and amide groups, Van der Waal interactions in the short chain amino acids are found between the molecules [13]. Glutamine, glycine residues in the amorphous regions contribute to the hydrophilic nature of the spider silk [75].

Table 2 shows amino acid contents of dragline silk and comparison with other protein fibers like wool keratin, silkworm silk [80]. In addition to the fibroin, other classes like glycoprotein, inorganic salts, sulphur containing compounds, amino acids, and ionic forms of amines are also present in the spider silk [69, 70]. Presence of these chemicals play crucial roles like identification of species, regulation of water content of the web, protection against microorganisms. Presence of 12-methyltetradecanoic acid and 14-methyl hexadecanoic acid to the minor amounts impart antimicrobial properties to the spider silk. Wax like esters are also present in the surface of the spider silk.

Action of solvents, Supercontraction

Spider silk is resistant to the most of the common solvents and enzymes except chymotrypsin. The spider silk protein is partially soluble in mineral acids and alkaline hydroxide and completely soluble in concentrated solutions of LiBr, Lithium thiocyanate or CaCl₂, ethyl alcohol, mercapto ethanol, and mixture of boiling HCl and propionic acid [35, 81, 82]. Unrestrained major ampullate fibers undergo supercontraction up to 60 – 80% in the presence of NaSCN, urea, which also increases with the polarity of the solvent e.g. CH₃OH, H₂O and results in reduction of modulus values, breaking strength. [25, 81, 82]. This type of contraction is not observed in minor ampullate silk, viscid silk to this extent [74, 82]. Supercontraction of dragline silk also takes place in air at about

an RH% of 90 or more and takes up slackness of the webs, restores shape and tension after prey capture [73, 83, 84, 85]. The super contraction ratios have been found to be 0.55, 0.82 for *Araneidae* and *Nephilengys* respectively [86]. Super contracted fibers can be re-extended to their original length in the same medium or in water.

Contraction leads to changes in conformations and orientation of the molecular chains due to rupture of hydrogen bonds [87, 88] and the deformational energy is stored in the form of conformational entropy. Initial exposure of spider dragline to moisture leads to development of supercontraction stresses, which reaches initially the yield stress and increases up to 22% of breaking stress depends on the effect of moisture on chain mobility, the rate of moisture enabled conformational changes, and the rate of stress relaxation of super contraction [73, 89].

Supercontraction affects the morphological parameters as well as the physical properties of the dragline silk [31, 35, 74, 76, 82, 84, 87 - 95]. In *Nephila* substantial fraction of the glycine glutamine, tyrosine, serine and leucine exhibit increase in molecular motions than polyalanine beta sheet regions [76, 82, 95]. Supercontraction indicates an increase in the random coil conformation with increase in elasticity, decrease in strength [76]. Supercontraction does not change the X-ray diffraction angle of the (200), (120), (210) reflections but long period is reduced from 8.4 to 5.8 nm [74, 90, 93] and crystalline orientation is reduced [92]. Influences of supercontraction over diameter, birefringence, and force-elongation relationship have been studied for various species of spider silk [21] and are mainly attributed to the plasticizing effect of the solvents. In the case of *Nephila*, the birefringence of the core does not change even after contraction but that of the skin is reduced to the half i.e. to 0.016 [92].

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Macroscopic Structure of Dragline Silk

Dragline spider silk is golden yellow in color and circular in cross section with a mean diameter about 7 μm [19, 38, 54]. No sericin or glue-like protein, similar to that of silkworm silk, is associated with dragline fiber [56]. Structural investigations of dragline silk reveal a skin-core structure with a weak skin [96 - 99], which is possibly due to the two different secreting regions involved in the production of the silk filaments. The diameter of the silk laid by the spider has the correlation to the weight of the spider in a small range [20]. Dragline silk consists of twin filaments with circular cross section, stuck together and aligned parallel to the filament axis [100]. Significant variation of the fiber diameter exists, along the fiber length because of the way in which silk filament is produced and laid, to the extent of $\pm 20\%$ of the mean value [99, 101, 102]. The density of the spider silk is about 1.33 - 1.35 g/cc [19, 52, 103], which is similar to that of other protein fibers like wool, silkworm silk but lower density values also have been reported in the literature [67]. The weight of female *Nephila* increases exponentially from juvenile to mature in autumn and the density also varies during these periods from 1.271 g/cc to 1.289 g/cc [100].

Outer layers of *Nephila madagascariensis* show a thin peripheral layer with elongated cavities like, caniculus, oriented parallel to silk fiber axis. These domains act as stress concentration points, crack initiators that inhibit the spreading of the cracks by delaying the rupture of the fiber by taking up the energy [98, 104]. The inhibition effect of these domains arise from self repairing of cracks by solidification under high stress areas, plasticizing effect by hydration to act as fluid shock absorbers, and act as a lubricant to reduce the inter fibrillar friction.

Fine Structure of Dragline Spider Silk

The dragline spider silk consists of semi crystalline polymeric structure with number of small crystallites between amorphous region [3, 14, 26, 50, 68, 73 - 76, 78, 94, 92,

105 - 114]. The alanine-rich regions (40%) are located in the crystalline domains of the silk fiber with beta sheet structure and about 60% of alanine is found in the weakly oriented and unaggregated beta sheets [73, 74]. In dragline, the glycine-rich chain segments adopt less ordered secondary structure in which helices can occur locally, stabilized by hydrogen bond. Glycine residues are partially found in beta sheets but contribute to helical structures showing 3-fold symmetry [50]. The presence of bulky glutamine residues limit the growth of beta sheets and force the formation of loops, tie chains and the surrounding amorphous matrix [72]. Poly (Gly-Ala) region can form a similar structure with lesser interactions, leading to lower tensile strength, which happens in minor ampullate silk. Disordered regions of major ampullate silk, in the relaxed fibers, follows the helical conformation under tension [106, 107].

Under tension, the glycine-rich sequences are able to form large-scale beta sheet crystal structures that are less perfectly ordered than the polyalanine crystals. The composition of repeat units varies to the extent of few nanometers, resulting variations in intersheet spacing and order and is termed non-periodic lattice crystals (NPL) [3]. Poly-alanine crystals are surrounded by a 'non-periodic crystal lattice' formed in the glycine rich sequences [74]. The crystal form of dragline silk takes different unit cell dimensions in their water-soluble and in-soluble form after spinning, though both are orthorhombic structures. The transition of soluble form into insoluble form involves reduction in the distance between the overlaying sheets to an extent of 18.3%.

Beta sheet conformations of the protein macromolecules lead to the formation of crystallites of at least 2 x 5 x 7 nm [68, 74] determined from the (002), (200), and (120) peaks. The diffraction pattern obtained from a single silk of *Nephila clavipes* yields reflections, showing orthogonal unit cell of a = 0.93 nm, b=1.04 nm and c=0.70nm [111]. SAXS shows an axial period of $\sim 79 \text{ \AA}$, and the smallest ordered regions exist in the

range of 20 \AA across [3, 109]. TEM results show higher domain size of the crystallites i.e. 70 – 500 nm including non periodic lattice regions [76]. The degree of crystallinity is about 30%, which exist in both the oriented state and partially oriented state [14, 110, 112, 113] with the orientation function of 0.776 - 0.979 [68, 111]. The variation of reeling speed of silk by spiders apparently does not influence the lateral packing of the molecular chains and the size of the crystallites, however, it alters the azimuthal width of the crystallites. Post spinning drawing of silk obtained from 'forced silking' process results in the partial degradation of crystalline region with improved amorphous orientation [115].

In general the fine structure of a fiber may be described using one phase model with continuous defects in a regular oriented system or two-phase model with separate crystalline and amorphous regions. However, in many occasions, these models become inadequate description for explaining the properties of the polymers,

fibers, and composites (Fig. 3), which in turn require an additional phase "inter phase", a morphologically distinct region, which is also known as intermediate phase or oriented amorphous phase [116, 117, 72]. This intermediate phase has physical properties that are different from both amorphous and crystalline phases. Formation of interphase takes place in a polymer when the orientations of the crystallites are not convenient for the re-entry of the chains and when the crystallites are small the formations of inter phase increases e.g. dragline silk. Typically, the crystalline fraction of the dragline silk varies from 0.1 to 0.4, which does not support the mechanical properties and necessitates an additional interphase, which may vary from 1-4 nm around every crystallite [116]. The density of interphase is in the range of 1.145 to 1.157 g/cc, which is different from the amorphous state (1.139 g/cc). Also, the glass transition temperature (T_g) of the inter phase is higher than that of amorphous phase with the modulus around six times higher than the amorphous state.

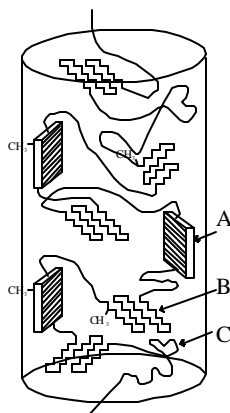


Fig. 3 Secondary Structure of Dragline Silk

A – Crystalline region, B – Oriented amorphous region, C – Amorphous region

Optical Properties

Optically isotropic protein secretions of MA glands are transformed into anisotropic liquid crystals and perfect three-dimensional crystallites in the extrusion process of the silk protein [3, 56, 57, 60, 68, 73, 74, 109, 111]. The birefringence of *Nephila clavipes* liquid protein is low to the extent of 0.01 to 0.002 and the contribution of form

birefringence to the total birefringence of the fibers has been found to be more significant. Insects are attracted to the web due to the attractive pattern and light reflecting properties of the web [118]. The silvery carapace of the *Nephila* helps to reflect sun light, which reduces the over heating of insects. Light reflection properties of the spiders, webs contribute to maintain the physiological conditions and prey capture.

Spiders and their webs are capable of protecting themselves from adverse conditions and overheating due to sun light [34, 119]. Forced “evaporative cooling” in the body also takes place above 35° C. Definite dark and bright patterns existing over the body of the spiders also help them to camouflage themselves from other insects and predators [120, 121]. Tiny scales on the bodies of the matured male jumping spiders reflect UV light, to identify and communicate with other spiders [122]. Brightly colored *Nephila*, spiders with both bright and dark markings are able to attract more insects through their ultraviolet reflecting properties. The webs, with the reflection patterns that resemble many flowers in UV light, attract and trap the insects than unreflected portions [34, 123].

Mechanical Properties

The mechanical properties of the dragline silk are highly influenced by the composition of amino acids, insect size, diet conditions, body temperature and drawing speed [99, 124]. Dragline silk, frame thread should be tough enough to be an energy absorber in order to catch the insects and help the spiders to escape safely. The sensitivity of the spider silk to humid conditions necessitates controlled environment for performing various tensile tests. With increasing relative humidity the stress developed in the fibers increase very significantly with decrease in yield stress [94, 124]. Stiffness, strength, and toughness of the dragline silk increase with the strain rate, which is an essential criterion in the prey capture or during the fall of the spider [74].

The stiff radial threads have time-dependent visco-elastic properties while the pliant capture threads have time-independent elasticity [24]. Stress relaxation in the silk fibers depends, largely, on the initial stress level applied to the fiber and up to 45% decay in stress levels are obtained if the fibers are allowed to relax within the yield stress [94]. In the orb-webs, the longitudinal waves are transmitted with little or no

attenuation and resonance effects are not found in the frequency range at which the preys are expected to produce the impact [125]. Energy dissipation through stretching and relaxation of the web is too weak, however, the main importance of the web lies in the dissipation of the kinetic energy of the insect upon impact on the web. Similar to high performance fibers, the molecular deformation pattern of *Nephila edulis* reveals a linear relationship in shifting of wave number in Raman Spectroscopy [103, 126].

Structural analysis of spider dragline silk carried out through Atomic Force Microscopy reveals a fibrillar structure with pleated fibrils at the core of the fiber, which can be unfolded with the extension [127]. Spider silk displays a force-extension curve that rises to a large force quickly and maintaining it over large extensions [128]. Stretched fibers usually snap back because the surface crack propagates only up to the point in the crystallites [19]. Measurement of modulus values have been found to be difficult on account of wide variation in diameter of the fibers [52] and the initial modulus of *Nephila* silk is close to 12.70 GPa with an elastic strain about 3.0 % [28, 100].

The breaking strength of silk increases linearly with increasing spider weight [47, 100, 129] and breaks at a stress about six times the spider’s weight. The stress-strain curve of dragline enters non-linear region when the stress exceeds twice the spider’s weight. The average tensile strength of the dragline of *Nephila clavipes* is almost three times that of *Bombyx mori* (1.3 & 0.5 GPa, respectively) with the elongation at break of 40% and 15% respectively [14, 37, 100, 102, 112]. The highest strength of dragline silk has been measured for *Nephila clavipes* (1.7 GPa to 2.9 Gpa) and lowest for *Araneus diadematus* (0.8 Gpa) [77, 130 - 132]. Tensile strength of spider silk reduces, when it is subjected to acid rains, UV radiation [133].

Spider silk exhibits a high level of torsional resistance, with a shear rigidity of 2.38 GPa

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which is higher than all textile fibers [28]. Higher torsional stability is essential in the case of dragline to serve as the life-line for the spiders. Spider silks can undergo large tensile and compressional deformations (20 - 40%) without any evidence of fracture or kink formation [28, 134, 135]. The compressive modulus has been found to be around 0.58 GPa. The ability of spider silk to resist transverse compression is lower than many of the textile fibers like Kevlar 29, nylon 5, polyester and wool.

Wide difference exists in terms of breaking energy between both the silks (16×10^4 and 6×10^4 J/kg respectively). Several types of fracture behavior exist [97] in the spider silk viz., brittle failure at one place, similar to that occur in ceramics, multiple brittle failure, and failure with elongation. Ductile failures at low strain rates are also observed in the dragline silk of *Nephila clavipes* [131].

Thermal and Electrical Properties

A very low second order transition, high degradation temperatures and sustained properties at extreme temperatures make the thermal properties of spider silk more interesting [13, 52, 136 - 138]. Chemical composition and arrangement of the structural elements of the silk protein has been used to predict the thermo-mechanical properties of the fibers. The glass transition temperature of the spider silk lies in the range of -50°C to -60°C and the transitions observed at -20°C and 30°C remain still unexplained [137]. Thermal expansion coefficient (α) value of dragline silk has been found to be $-10.6 \times 10^{-4} \text{ }^\circ\text{C}^{-1}$ [24], which reduces further with the increasing draw rates. Molecular organization of *Araneus diadematus* silk fibroin is unaffected by exposure to 100°C for over 50 h [136, 139] and the functional properties are retained up to 180°C without affecting the initial modulus. Thermal decomposition occurs above 250°C and the fibers are thermally stable up to 230°C [13].

Many natural biopolymers, like, wood, wool, silk fibroin and collagen display

piezo-electric effect. Piezo-electricity is the consequence of dipole orientation in polarized species containing carbon, oxygen, nitrogen and hydrogen [137]. Piezoelectricity of spider dragline is minimal compared to other natural polymers. In the randomly distributed dipoles, no piezo-electric is observed.

REGENERATED SPIDER SILK

Forced Silking (Reeling) of Spider Silk

Reeling devices have been developed for forced silking of dragline from the glands of anaesthetized *Nephila clavipes* [78, 115, 140, 141] to reel about 3-5 mg of silk in one session. In forced silking under anesthetized conditions, the cross section of the filament changes from circular to ellipsoid with variation in diameter along the length [12, 102]. But forced silking of fibers, without anesthetic condition, result in properties similar to that of normally spun spider silk [12].

Spider silk protein – Artificial route

Protein of dragline has attracted many researchers on account of its superior properties [142 - 147] and details of structure and expression of silk proteins of both silkworm and spider have been reviewed along with the genetic engineering aspects [18, 49, 145, 148]. Splicing of silk genes into two different cell lines have been tried in the past using bovine mammary cells and hamster kidney cells, to produce large volumes of recombinant proteins [49, 135]. Successful sequencing of genes of the flagelliform silk of tropical spider *Nephila Clavipes* and *N. Madagascariensis* has been achieved very lately [39].

A method has been developed to isolate cDNA, capable of expressing spider silk proteins, and for producing recombinant protein [149]. Recombinant DNA technology for microbial proteins [144, 150, 151] appears to be advantageous compared to that of chemical synthesis due to low cost, rapid preparation and absence of byproducts. In spite of numerous steps involved in

chemical synthesis, the yield and molecular weight seems to be very low with high polydispersity, whereas biosynthesis of polypeptide molecular weight to the extent of 40,000 with monodispersity could be achieved [144]. Various recombination techniques for protein synthesis have been successfully reported using microbial sources [145, 152, 153] transgenic techniques involving mammary cells [154 - 158], plant cells and leaves [151, 159]. Table 3 summarizes the various attempts in recombinant synthesis of spider silk protein.

From the known sequence of the spider silk protein, genes are constructed and expressed using *E. Coli* as the host, which has been successfully used earlier for silkworm silk [160] also. *E. Coli* as the host yields the protein to the extent of 10 mg/g in the wet state [161 – 163] and 1-2 mg/g of *E. Coli* after purification, which is higher than that obtained using *Bascillus subtilis* [152, 164].

Genes of spider dragline silk have been inserted into mammary gland cells along with regulatory elements. Insertion of the genes into single cell goat eggs has been tried to produce water-soluble silk protein

[154, 156, 165]. Few milligrams of genetically engineered silk like protein have been successfully produced based on the sequence of spider protein [155]

Recombinant proteins of *Nephila clavipes* using gene synthesis route have also been attempted in endoplasmic reticulum of tobacco leaves, and potato leaves and tubers. Such proteins shows excellent heat stability while purification [159] and is relatively cheaper to produce than bacterial method.

Producing recombinant silk proteins is relatively easier than regeneration, spinning and drawing which are the bottlenecks in the whole process sequence [142]. The protein sequence of *Nephila clavipes*, synthesized by cloning shows high susceptibility to enzymatic cleavage near the inverted repeats [143]. Upon purification, the recombinant protein rapidly self-assembles to form insoluble micro fibrils and poses a limitation in the subsequent conversion into like fibers. Self “sterical-triggers” are introduced into the system to control the conformational changes by preventing hydrophobic interactions between the overlaying sheets e.g. redox triggers [145, 166].

Table 3. Different hosts and their origin used for spider silk synthesis

Origin	Host type	Product, Yield	Ref.
Microbial	<i>E. Coli</i>	Not available	145
Chemical method	---	MW – very low	147
Microbial	<i>E. Coli</i>	MW – 40000	147
Microbial	<i>E. Coli</i>	10mg/g	157
Mammalian cells	Goat cells	Not available	160
Plant leaves and tubers	Endoplasmic reticulum	2% accumulation	162
Microbial	<i>E. Coli</i>	10 mg/g	164
Microbial	<i>E. Coli</i>	More than 1mg/g	167
Microbial	<i>E. Coli</i>	Low	169

Regeneration or Spinning

Table 4 summarizes various attempts reported in the literature to regenerate the silk proteins into fiber form [155, 164, 167, 168]. Solution spinning of silk protein in different solvent-non solvent systems like hexafluoro isopropanol HFIP –isopropanol

[164], HFIP – methanol [167], guanidine-hydrochloric acid mixture followed by room temperature drying [169] have been attempted using special spinning set up.

A biomimetic rig to imitate the biological set up [48] and electro spinning method using a blend of PVA and silk protein (50/50

by weight) with 35 kV have been tried for the production of drag line [137]. Etched silicon wafers with the aperture sizes of 80 – 160 μm and syringe have been successfully used to extrude the filaments from protein solutions of dragline silk with a diameter of 20 to 80 μm and surface characteristics similar to the native filaments [155, 164, 170]. The wider variation in the diameter is attributed to the uncontrolled reeling of filament in plastic condition. Blunt point hypodermic stainless steel needles with a diameter of 150, 175 and 250 μm attached to disposable syringes have also been used [170, 171] as spinnerets for producing silk fibers. The filaments are subsequently drawn in air at 8 cm/sec under the wet condition followed by drying under tension and the filaments appear to withstand a total draw ratio of 9 in two steps without rupture. Microscopic analysis of freshly regenerated fibers exhibits low-density values on account of voids created due to the transfer of solvent, which remains even after second drawing, while spongy appearance is modified into the fibrillar structure. The highest strength measured in the regenerated and drawn dragline silk fiber remains about 320 Mpa with the modulus value of about 8.0 GPa compared to 875Mpa and 10.9 GPa of native fibers used for regeneration. The fraction of alanine β sheet structure is found to increase linearly with the extent of drawing applied to the specimen [172]. The two stage drawn

fibers exhibit fraction of β sheet conformation as high as 65%, which is closer to the native fibers. While no preferential molecular order is observed in as-spun fibers, significant crystalline alignment with respect to the fiber axis is achieved when the spun fibers are stretched in water for the second time. The drawn fibers exhibit low average orientation than the native fibers due to the presence of unoriented crystalline regions.

Fibers regenerated from proteins of *Nephila edulis* spider shows a diameter of 9 μm and rough surface along with wrinkles. An initial modulus of 6.0 GPa and breaking strength of 0.11 to 0.14 GPa with the elongation at break of 10% to 27% has been reached in this case. The values appear to be much lower than native spider silk except for the elongation at break [173]. Regenerated spider silk exhibits dichroism only after drawing, indicating the random distribution of ordered beta sheets in the unstretched filament.

The regeneration of spider silk proteins leads to the conformational changes back into original structure upon spinning. However, the re-conversion happens only to a lesser extent and obtaining higher beta sheet conversion during spinning and post spinning operation is very much essential in achieving better properties.

Table 4 – Properties of regenerated spider silk

Solvent-non solvent system	Concentration (%)	Fineness	Tenacity	Elongation (%)	Initial Modulus	Ref.
HFIP-Isopropanol	20	16.7denier	1.22 g/d	103.3	40 gpd	167
Guanidine-HCl mixture	0.08	9 μm	0.11-0.14GPa	10 - 27	6.0 GPa	171
HFIP - acetone	2.5	20 μm	320 MPa	4 – 8	---	172
HFIP- acetone	2.5	20–80 μm	NA			155
Natural spider silk	30%	> 2.7 μm	1.3 – 2.9GPa	40%	60 GPa	37, 102, 115,116

APPLICATIONS

Though availability of the dragline silk is very much limited, it is widely used in defense [4, 131, 140, 174, 175] and medical [11, 19, 49, 100, 105, 141, 151, 176, 177,] applications and potential applications have also been discussed in the past [100, 178, 179]. Structural similarity and comparable properties of dragline to that of high performance fibers like Kevlar [127, 180] makes it more attractive for applications where high performance, in terms of physical properties are demanded.

Until World War II, spider silk was used as crossed-hairs in optical devices including microscopes, telescope and bomb guiding systems [4]. Silk strands of the web have an ability to elongate when an insect is caught, convert the prey's momentum i.e. kinetic energy into heat, and dissipate about 70% of the converted energy. The web also gently rebounds so as not to catapult the insect back out. This ability to dissipate energy at very high strain rates makes spider silk suitable for body armor system and ideal for ballistic protection [131, 140]. Though biodegradability is a helpful aspect for sutures but is as unwanted one high performance applications such as bulletproof vests. A very low glass transition temperature of -50°C to -60°C enables it to absorb sudden shocks at low atmospheric temperature and makes the spider silk suitable for parachute applications. However, super contraction in water is undesirable for use in the fabrication of parachutes [174, 175].

Earlier use of spider silk in the form of web, rather than a fiber, includes wound dressing to help blood clot and fishing nets. Biomedical application of silk materials, such as a degradable suture has been reviewed earlier [11, 49, 100, 105, 141, 146, 176, 177, 181, 182]. Spider silk protein can be used to coat the medical implants for better performance. Surgical thread, biomembranes and scaffolds for tissue engineering are the possible areas of application in biomedical and biomaterial

fields due to low inflammatory potential of silk proteins and antithrombic nature. Recombinant spider silk has potential applications in sutures for eye surgery, artificial tendon, ligaments for knee construction.

Spider silk with higher safety co-efficient can be used in structural applications like elevator ropes, bridges and pillars [100]. Spider silk can be used as reinforcement for other fibers and can be incorporated directly as it is spun from the glands of the *N. clavipes* [178]. In another application, spider silk of *Nephila madagascariensis* is repeatedly treated with tetraethyl orthosilicate and then allowed to dry followed by baking at 420°C to burn away the silk. This results in shrinkage of silica to form hollow silica tube with a diameter of just $1\ \mu\text{m}$, which can be used as hollow optical fibers to carry light beams in the nanoscale optical circuits or as nanoscale test tubes. These narrow fibers can also be used as fiber optic probes for near field microscope [179].

CONCLUSION

The dragline silk offers excellent physical and chemical properties that can withstand the adverse and extreme conditions than many of the existing natural and synthetic fibers. Though the chemical synthesis seems to be unfruitful in many aspects, the recombination method of producing the spider silk using biological hosts proves to be a viable option for producing the spider silk in a large scale. In spite of various successful attempts made in the production of dragline silk in the laboratory scales, controlling the molecular conformation and their aggregation during the spinning for achieving properties similar to the native fiber still remains as a challenge for the future research.

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