

A REVIEW ON SPIDER SILK STRUCTURE, FUNCTION AND APPLICATIONS

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Abstract: Spider silk is an excellent biomaterial with outstanding mechanical stability, elasticity, and biocompatibility. Although promising, the cannibalistic nature of spiders poses a challenge to large-scale production, hindering commercial exploitation. Various silk varieties produced on spinnerets have acquired considerable importance, especially that of *Trichonephila clavipes*, which belongs to the genus *Nephila*. This review emphasizes the biochemical structure of dragline silk and discusses its ultrastructure using various microscopic techniques to elucidate the hierarchical structure of this substance. Furthermore, it also highlights the extensive applications of spider silk, particularly in biomedical, industrial, and technological fields. Integrating structural and functional perspectives, this review emphasizes the potential of dragline silk as a model biomaterial for future translational research.

Keywords: biocompatible; biomaterial; dragline silk; microscopic; spider silk

1. Introduction:

Silk is a broad term used to refer to continuous fibres spun by various species of Lepidoptera, viz., butterflies, moths, and arthropods, such as arachnids, and it is utilised for various purposes, such as catching prey and web construction [1]. The silk produced by *Nephila* spiders is seen to possess better qualities. Numerous spider species exhibit remarkable diversity in their webs, silk glands, and silk fibre properties, which are closely related to their lifestyle and diet. They produce webs that vary in colour, strength, and composition, reflecting adaptations to their prey-capture strategies and habitat [2]. Spiders have distinct sets of glands, each specialized in crafting a distinct type of fibre. In the early stages of spider evolution, these glands did not have specific functions. [3]. Spider silk is produced by various spider species worldwide, with *Nephila sp.* known for creating the finest and most mechanically robust silk [4].

Spiders weave different types of webs depending on their species, and many spiders are classified based on the webs they weave. Orb webs are associated with the families Araneidae, Tetragnathidae, and Uloboridae, whereas, tangle webs and cobwebs are primarily linked to the family Theridiidae. Funnel webs are associated with primitive and modern species, tubular webs are linked to species that go up tree bases or spread along the ground, and sheet webs [5], respectively. Species from different families, such as *Araneus* and *Nephila*, construct orb webs and produce silk.

Silk proteins exhibit divergent amino acid compositions, which aid in folding, silk strength and maintaining higher extensibility. To infer the structural organization of the native thread, a comparative microscopic study was conducted on *Bombyx* and *Nephila* to understand their supreme tensile strength, which may be important for web functionality [6]. This review aims to understand the biochemical and microscopic properties of silk from *Trichonephila clavipes* and its applications, owing to its superior properties and great strength.

2. Golden Orb-Weaving Spiders:

Nephilids can grow to a body length of 5 cm and boast a leg span of 15 cm. They are common and spectacular inhabitants of tropical and subtropical regions [7]. *Nephila* females spin some of the largest orb webs recorded to date, with a diameter of 1.5 m and unique golden silk [7]. *Trichonephila clavipes*, previously identified as *Nephila clavipes* and a member of the Nephilinae family, is commonly employed in studies focusing on silk glands and the practical uses of silk products. Spiders of the subfamily Nephilinae and the genus *Zygiella*, which were previously classified within the family Araneidae, have recently been reassigned to the family Tetragnathidae. Both families belong to the Araneoidea superfamily [8]. Golden orb-web spiders of the genus *Nephila* are distributed throughout the tropics [9], and exhibit extreme sexual size dimorphism [6]. *Nephila clavipes*, also known as *T. clavipes* or the banana spider, constructs large, asymmetrical circular webs attached to trees and low shrubs in woods to catch small and medium-sized flying prey, primarily insects.

3. Spider Silk and silk-producing glands:

Orb-weaving spiders possess up to seven distinct types of glands that produce silk for their webs, including major ampullate, minor ampullate, pyriform, flagelliform, aggregate, tubuliform, and aciniform. The primary elements of each silk gland are spider silk fibroins, commonly known as spidroins, which derive their names from the specific gland where they were first identified [10]. Minor ampullate and viscid silks are the predominant silk and the spider dragline appears as anchoring threads, which form the architecture and pretensioned radial filaments dominate the web structures [1]. Dragline silk is aromatic, which helps spiders to breed, wander, stockpile food and eggs, and facilitate reproduction [1]. Minor ampullate silk, produced by the median spinneret, is characterized by high elasticity and low strength. These silks are finer than major ampullate silk and demonstrate better uniformity, although their properties remain uncertain. Dragline silk, sometimes called lifeline silk, is produced in the major ampullate silk region of spiders and is assembled from structural proteins called spidroin. These silks are thinner than major ampullate silk, and are more homogenous though their properties are still not clear [11]. It is an extracellular fibrous protein produced with unique strength and elasticity characteristics, and its toughness exceeds that of all other known natural materials [12]. Measurements of reeled dragline silk indicate that it is strong enough to support the spider and prey that doubles its weight. Multistrands of a spider dragline can be created by passing through the same route many times. Furthermore, orb spiders can produce multi-stranded threads by traversing the same path multiple times [11]. Spider dragline silk is significant because of its high strength and elasticity. An overview of mechanical and functional properties of these silks is given below in order to increase the clarity:

Table 1: Mechanical and functional properties of spider silks [10]

Silk Type	Gland	Function	Key Mechanical Property
Dragline	Major ampullate	Radial/frame threads, lifeline	High strength, high and high toughness
Minor ampullate	Minor ampullate	Temporary scaffolding	Moderate strength, high elasticity
Capture spiral	Flagelliform/aggregate	Prey retention, extensible spiral	Very elastic, sticky
Egg sac	Tubuliform	Egg protection	Moderate strength, durable
Prey wrapping	Aciniform	Prey immobilization	Tough, flexible

4. Biochemistry and Microstructure of Spider Silk:

Spider silk is mainly composed of proteins containing non-polar and hydrophobic amino acids such as glycine and alanine, but lacking tryptophan. The protein structures of silk have a highly divergent amino acid composition compared to typical cellular enzymes [13]. The major ampullate silk of *T. clavipes* is composed of two substantial proteins, each approximately 250-350 Da in size, known as major ampullate spidroin1 (MaSp1) and major ampullate spidroin2 (MaSp2), which are distinguished by their diverse amino acid compositions.

Major ampullate spidroin is a complex of rigid nanocrystals that provides strength and extensibility to the fibres [13]. Researchers have established a high alanine and glycine content in Masp1 and almost no proline in MaSp2. The high glycine and alanine content of refined MaSp1 is especially notable in the process of creating the 80-sheet crystalline region of silk fibres, which makes them stronger and firmer [15]. MaSp2, with its high concentration of prolines, is primarily located in the amorphous spaces between the 2-sheet crystals, enhancing the extensibility of the fibres [14]. Specifically, the amino acid content of dragline silk is glycine (43.9%), alanine (29.69%), small chains (62%), proline (14.22%), serine (5.1%), and glutamine (11.29%) [17]. Moreover, highly repetitive sequences of amino acids, particularly in the large core region, have been observed in spider silk proteins. The repetitive sequences consist of short polypeptide extensions, ranging from approximately 10 to 50 amino acids, and account for over 90 percent of the entire spider silk protein. These motifs may occur in a single protein over a hundred times [18]. All polypeptide repeats possess unique functional properties that contribute to the exceptional mechanical characteristics of spider silk threads. In addition to the repetitive core domain, non-repetitive areas are found at the ends of the protein. The unique terminal regions of spider silk proteins are very essential for fibre formation [19]. Once the silk gland secretes silk, the silk proteins in the aqueous solution initially lack significant secondary or tertiary structures. It is interesting to note that it has long repetitive sequences in their central domains, which enable weak intra and intermolecular interactions of neighbouring domains and proteins in their passage through the spinning ducts. These relations eventually give rise to secondary, tertiary and quaternary protein structures. Phosphate ions are necessary for spider silk protein self-assembly and for inducing structural changes. Phosphate ions have been reported to create liquid-liquid phase separation in spidroin solutions (LI), leading to the precipitation of droplets of protein-rich solutions [20]. The hierarchical self-assembly of silk

fibres was initiated by these droplets. Phosphate ions preincubate spidroins, facilitating the formation of β -sheets, which is crucial for creating strong silk fibres. Additionally, amino acid structures may differ from those of the foods they consume. The dragline silk of *Nephila* that consumes crickets contains a higher percentage of proline and glutamine than that of *Nephila* that eats flies, with a much higher percentage of alanine [21]. The lipid composition of the dry fibre of *T. clavipes* dragline is lower and ranged from 3-5 percent, which may be due to deposition on the web while the spider walks. Gas Chromatography-Mass Spectrometry (GC/MS) detected the presence of hydrocarbons, alcohols, and unique methyl-branched alkyl methyl ethers. Subsequent discussions revealed that alkanes were the second largest group of substances in the silk extracts. Fatty acid analysis of the extracts revealed that trimethyl sulfonium hydroxide produced fatty acid methyl esters and methylated alcohols [22]. The existence of pyrrolidine in the dragline silk of *Nephila* is hygroscopic, which maintains skin moisture. KH_2PO_4 is also significant because it helps prevent fungal and bacterial infections. It exudes hydrogen ions in water solution, which causes a pH of 3, making silk very acidic and ultimately killing bacteria and fungi [23].

Microscopic examination of the dragline silk of *T. clavipes* revealed that the silk thread contained an unknown organization of β -sheet crystals. Using scanning electron microscopy (SEM), researchers have identified the presence of a tube within the dragline thread, a discovery further corroborated by light microscopy studies. They also showed that the tube contains a less dense core material with twisted bands of microfibrils, and an outer coating and inner membrane cover the tube [6]. Atomic force microscopy (AFM) studies showed that the topography of the spider dragline silk had roughness on its surface, alternating with smooth surfaces, superimposed light and dark color nanostructures indicating low and high features made of β sheets, β turns, and α helices, which give a semicrystalline nature to the silk. Furthermore, roughness analysis showed that the roughness values changed with the scan size, because the biocompatibility of the material is dependent on surface roughness, which imparts high and stable mechanical properties to spider silk [24].

5. Applications of spider silk:

The special properties of spider silk as a natural biomaterial have been applied in many applications because it is biodegradable, lightweight, highly biocompatible, and has unique mechanical properties. However, several studies [25] have recognized that most silk materials used in biomedical applications have appropriate mechanical and physical qualities, but lack biocompatibility and antibacterial properties. Therefore, the extraction of spider silk, particularly dragline silk, is necessary because of its low inflammatory properties and biocompatibility. Dragline silk has various medical applications, including use in surgical threads, biomaterial membranes, scaffolds, substrates that support cell growth, 3D printer bioinks, and controlled release matrices, due to its low potential to cause inflammation [26]. Other characteristics of spider silk, such as low homogeneity, hydrophobicity, and adjustability, have drawn much attention in drug and gene delivery applications [27]. However, the existing uses of silk are in silkworm fibroin because spider silk is not sufficiently available. Recently, the silk of *T. clavipes* has been explored to determine its utility for surgically improving mammalian neuronal functions. *In vitro* experiments showed that a filament of the silk can lead a severed neuron through the body to the site of severance. Silk shows no reaction from the immune system and thereby escapes rejection by

the host body [28]. The dragline silk of *T. clavipes* has efficient optical properties and is used for biosensor systems [29] as well as for industrial purposes, such as bulletproof clothing, wear-resistant lightweight clothing, ropes, nets, seat belts, parachutes, and corrosion-resistant panels on motor vehicles or boats [30].

6. Conclusion:

This review highlights different silk-producing glands, focusing on the dragline silk of *T. clavipes*, its biochemical and microscopic properties, and its applications. Dragline silk is one of the toughest silk forms in orb-weaving spiders, and studies on the biochemical composition of dragline silk suggest that its amino acid profile is optimally rich in glycine (37%), with alanine following at 18%. According to several studies [17], the glycine-rich region in spider dragline silk contributes to elasticity, and the alanine-rich region forms β -sheet crystalline structures, highlighting their significant contributions to the mechanical properties of fibres. The increased proline content was indicative of relatively increased proportions of MaSp2, greater amounts of beta turn, and greater extensibility of the silk. In contrast, when the percentage of alanine in dragline silk is much greater, it implies that there will be a greater percentage of MaSp1 protein, more beta sheets, and a higher tensile strength [14,31]. Further studies have found that the pyrrolidine content of dragline silk has hygroscopic properties that keep silk moist. The presence of potassium hydrogen phosphate makes silk highly acidic, preventing further fungal and bacterial infections [21]. Thus, dragline silk is one of the most appropriate silks for various applications. Microscopic work clearly shows the structures of the thinnest piece of dragline silk. Hence, this review offers a clear understanding of the structure and composition of dragline silk, achieving a broad concept across various fields.

References:

- [1] D. Saravanan *et al.*, “Spider silk – Structure, properties and spinning,” *J. Textile Apparel Technol. Manag.*, vol. 5, no. 1, pp. 67–73, 2006.
- [2] R.S. Gole, R. S. and P. Kumar (2012). Spider’s silk: Investigation of spinning process, web material and its properties. *Biological Sciences and Bioengineering, IIT Kanpur, India*, 2012.
- [3] A. Rising, H. Nimmervoll, S. Grip, A. Fernandez-Arias, E. Storckenfeldt, D. P. Knight, *et al.*, “Spider silk proteins – mechanical property and gene sequence,” *Zool. Sci.*, vol. 22, no. 3, pp. 273–281, 2005.
- [4] K. Singh, S. Maity and M. Singha, Spinning and applications of spider silk. *Front. Sci.*, 18, 20, 2012
- [5] M. J. Roberts, *The Spiders of Great Britain and Ireland, Volume 1: Atypidae to Theridiosomatidae*. Colchester, England: Harley Books, p. 229, 1985.
- [6] F. Vollrath, T. Holtet, H. C. Thogersen, and S. Frische, “Structural organization of spider silk,” *Proc. R. Soc. B*, vol. 263, no. 1367, pp. 147–151, 1996.
- [7] J. D. Van Beek, S. Hess, F. Vollrath, and B. Meier, “The molecular structure of spider dragline silk: folding and orientation of the protein backbone,” *Proc. Natl. Acad. Sci. USA*, vol. 99, no. 16, pp. 10266–10271, 2002.
- [8] M. Kuntner and J. A. Coddington, “Discovery of the largest orb-weaving spider species: the evolution of gigantism in Nephila,” *PLoS One*, pp. 1–7, 2009

- [9] L. Römer and T. Scheibel, "The elaborate structure of spider silk: structure and function of a natural high-performance fiber," *Prion*, vol. 2, no. 4, pp. 154–161, 2008.
- [10] P. A. Selden, C. Shih, and D. Ren, "A golden orb-weaver spider (Araneae: Nephilidae: Nephila) from the Middle Jurassic of China," *Biol. Lett.*, 2011, vol. 7, no. 5, pp. 775–778, 2011.
- [11] Y. C. Su, Y.H. Chang, D. Smith, M. S. Zhu, M. Kuntner, and I. M. Tso, "Biogeography and speciation patterns of the golden orb spider genus *Nephila* (Araneae: Nephilidae) in Asia," *Zool. Sci.*, vol. 28, no. 1, pp. 47–55, 2011.
- [12] M. Branković, F. Zivic, N. Grujovic, I. Stojadinovic, S. Milenkovic, and N. Kotorcevic, "Review of spider silk applications in biomedical and tissue engineering," *Biomimetics*, vol. 9, no. 3, p. 169, 2024.
- [13] R. V. Lewis, "Spider silk: Ancient ideas for new biomaterials," *Chem. Rev.*, vol. 106, pp. 3762–3774, 2006.
- [14] F. Teulé, B. Addison, A. R. Cooper, J. Ayon, R. W. Henning, C. J. Benmore, *et al.*, "Combining flagelliform and dragline spider silk motifs to produce tunable synthetic biopolymer fibers," *Biopolymers*, vol. 97, no. 6, pp. 418–431, 2012.
- [15] M. Marhabaie, T. C. Leeper, and T. A. Blackledge, "Protein composition correlates with the mechanical properties of spider (*Argiope trifasciata*) dragline silk," *Biomacromolecules*, vol. 15, no. 1, pp. 20–29, 2014.
- [16] D. Huemmerich, C. Helsen, S. Quedzuweit, J. Oschmann, R. Rudolph, and T. Scheibel, "Primary structure elements of spider dragline silks and their contribution to protein solubility," *Biochemistry*, vol. 43, no. 42, pp. 13604–13612, 2004.
- [17] Z. N. Al Azawi, "Study on chemical composition and designing web patterns of Iraqi spider silk," *Sci. Rep.*, vol. 10, pp. 1–13, 2020.
- [18] J. Li, G. Z. Yang, X. Li, H. L. Tan, Z. W. Wong, S. Jiang, and D. Yang, "Nanoassembly of spider silk protein mediated by intrinsically disordered regions," *Int. J. Biol. Macromol.*, vol. 271, p. 132438, 2024.
- [19] T. Scheibel, "Spider silks: Recombinant synthesis, assembly, spinning, and engineering of synthetic proteins," *Microb. Cell Fact.*, vol. 3, p. 14, 2004.
- [20] L. Zeußel, V. T. Trossmann, C. B. Borkner, V. Beyersdorfer, M. Rottmar, and T. Scheibel, "Liquid–liquid phase separation of spider silk proteins," *Nature Mater.*, vol. 24, pp. 123–134, 2025.
- [21] D. B. Zax, R. V. Lewis, and M. B. Hinman, "Dietary effects on the amino acid composition of spider dragline silk," *J. Exp. Biol.*, vol. 207, pp. 4205–4212, 2004.
- [22] S. Schulz, "Composition of the silk lipids of the spider *Nephila clavipes*," *Lipids*, vol. 36, no. 6, pp. 637–647, 2001.
- [23] A. Lateef, S. A. Ojo, and J. A. Elegbede, "The emerging roles of arthropods and their metabolites in the green synthesis of metallic nanoparticles," *Nanotechnol. Rev.*, vol. 5, no. 6, pp. 601–622, 2016.

- [24] A. S. Sawarkar and S. B. S., “AFM study of egg sac silk fibers of the giant wood spider, *Nephila pilipes* (Araneae, Araneidae),” pp. 1–8, 2011.
- [25] S. Lentz, V. T. Trossmann, C. B. Borkner, V. Beyersdorfer, M. Rottmar, and T. Scheibel, “Structure–property relationship based on the amino acid composition of recombinant spider silk proteins for potential biomedical applications,” *ACS Appl. Mater. Interfaces*, vol. 14, pp. 31751–31766, 2022.
- [26] S. Salehi, K. Koeck, and T. Scheibel, “Spider silk for tissue engineering applications,” *Molecules*, vol. 25, no. 3, p. 737, 2020.
- [27] B. Bakhshandeh, S. S. Nateghi, M. M. Gazani, Z. Dehghani, and F. Mohammadzadeh, “A review on advances in the applications of spider silk in biomedical issues,” *Int. J. Biol. Macromol.*, vol. 192, pp. 258–271, 2021.
- [28] F. M. Chen and X. Liu, “Advancing biomaterials of human origin for tissue engineering,” *Prog. Polym. Sci.*, vol. 53, pp. 86–168, 2016.
- [29] Y. Q. Zhang, “Optical properties of spider silk and their potential applications in material and biosensor systems,” *Appl. Phys. A*, vol. 66, pp. S445–S448, 1998.
- [30] K. Singh, S. Maity, and M. Singha, “Spinning and applications of spider silk,” *Front. Sci.*, vol. 18, p. 20, 2012.
- [31] Y. Watanabe, “Molecular mechanisms of the high performance of spider dragline silk,” *Biomater. Sci.*, vol. 11, pp. 1–14, 2023.