With great structure comes great functionality: Understanding and emulating spider silk

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The overarching aim of biomimetic approaches to materials synthesis is to mimic simultaneously the structure and function of a natural material, in such a way that these functional properties can be systematically tailored and optimized. In the case of synthetic spider silk fibers, to date functionalities have largely focused on mechanical properties. A rapidly expanding body of literature documents this work, building on the emerging knowledge of structure–function relationships in native spider silks, and the spinning processes used to create them. Here, we describe some of the benchmark achievements reported until now, with a focus on the last five years. Progress in protein synthesis, notably the expression on full-size spidroins, has driven substantial improvements in synthetic spider silk performance. Spinning technology, however, lags behind and is a major limiting factor in biomimetic production. We also discuss applications for synthetic silk that primarily capitalize on its nonmechanical attributes, and that exploit the remarkable range of structures that can be formed from a synthetic silk feedstock.

I. INTRODUCTION

Silk is usually associated with fibers from the larval cocoons of the silkworm Bombyx mori, which are used in a range of applications from clothing to medical sutures. Spider silks, however, have been recently rising in prominence. Unlike silkworm silk, spider silk has been evolutionally driven for mechanical performance in webs and is the toughest biological material known.[1,2] The combination of impressive mechanical performance, green chemistry, biodegradability, and ambient processing conditions[3,4] makes spider silk a highly desirable material for applications ranging from biomaterials to high-performance industrial fibers. Despite its clear potential, it is extremely difficult to obtain silk from spiders,[5] and substantial research effort has been spent to produce spider-like silk at commercial scales by using biomimetic approaches. While we have not yet matched the properties of spider silk using scalable techniques, advances in our understanding of the structure and natural spinning of native silks, and improvements in protein expression, are driving the field closer to its goals.[6–9]

II. PROPERTIES OF SPIDER SILK

Spider silks possess a range of qualities that are rarely found simultaneously in one material. Toughness, extensibility, and strength are only a few of the desirable traits that make spider silk of so much interest as a potential commercial product. Silk is biodegradable and, unlike synthetic high-performance fibers such as Kevlar, it is extremely lightweight.[10–12] The mechanical performance of spider silk is retained over a large temperature range (−66 to 100 °C), exhibiting particularly interesting behavior at low temperatures. At the temperature of liquid nitrogen (−196 °C), silk has been shown to increase its strength by 64% relative to its strength at

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room temperature.[13,14] A further advantage lies in its processing conditions and requirements. Whereas the production of Kevlar and other such synthetics comes at a high monetary and environmental cost, spiders spin recyclable fibers on demand, under ambient conditions using water as a solvent, at the energetic cost of an insect dinner.[4,15]

The most studied and best understood spider silks are the major ampullate silks from the Nephilidae family of orb weavers. These are considered to be the benchmark for synthetic silks in terms of structure and mechanical properties.[3] In 2010, however, researchers described the toughest silk to date, from the Malagasy orb-weaver, Caerostris darwini (Darwin's bark spider).[16] At approximately 2 cm in length, Caerostris darwini is not an especially large spider, yet it is able to build webs across rivers.[17] In one observed case, the bridge line was 25 m across a lake, with capture areas up to 2.7 m^2.[17] Agnarsson et al.[16] reported the forcibly pulled major ampullate (Ma) Caerostris darwini silk fibers to be twice as tough as silks from other species, and 10 times tougher than Kevlar (see Table I).

Similar to many biological materials,[21] the outstanding performance of spider silks is the result of interactions between material building blocks over a range of hierarchical levels.[22–24] Extreme Poisson's ratios (1.29 and −0.48 for axial and radial deformation of N. clavipes silk) recently reported for major ampullate silks[25] reinforce the notion that spider silk should be viewed as a complex hierarchical structure rather than a continuum material.

The main functional characteristics of spider silk originate from interactions within and between two proteins: the highly ordered, hydrophobic Spidroin I (Sp1) and the less-ordered, hydrophilic Spidroin II (Sp2).[26,27] A range of order exists in the material,[28,29] predominantly in β-sheet, helical, and pseudoamorphous chain configurations[30] to form a complex, glassy polymer. The combination of these proteins can be simplified into ~40% ordered domains (two hydrogen bonds per amide group), ~15% permanently disordered domains (one hydrogen bond per amide group), and ~45% intrinsically disordered domains which can potentially become ordered.[31] The proline in MaSp2 is the best candidate for reversible disorder. It acts to disrupt secondary structure, twisting away from an ordered conformation, affecting the balance between entropic elasticity and bond-energy driven mechanisms of deformation and providing an important contribution to material properties at high humidity.[32–34] The reversible disorder from the presence of proline has been linked with sacrificial bonding mechanisms and high toughness in the inner core of the native silk fiber.[35] Through the manipulation of order, by modulating composition, environment or load history,[33,35,36] mechanical properties can therefore be tuned within a large performance envelope[37] to target applications requiring specialized mechanical characteristics. Recent experimental work[38] examining the propagation of acoustic waves in spider silk reveals that it can be tuned to support the largest range of longitudinal wave speeds of any material tested to date, extending possible uses to stimuli-responsive smart materials.

TABLE I. Comparison of typical mechanical properties of dragline silks from orb-weaving spiders with Kevlar and silkworm silk.

Fiber type	Stiffness (GPa)	Toughness (MJ/m^3)	Strength (MPa)	Extensibility (% strain)
Nephila clavipes[18]	13.8	111	1215	17.2
Gasteracantha cancriformis[18]	7.3	193	1199	33.1
Deinopis spinosa[18]	10.4	124	1345	19.1
Latrodectus geometricus[18]	10.2	117	764	31
Latrodectus hesperus[19]	10.2	181	1441	30.3
Caerostris darwini[16]	11.5	354	1652	52
Bombyx mori (silkworm)[20]	5	70	600	18
Kevlar[20]	100	30	4000	3.9

Beyond, but relating to an order-based view of mechanics, the high toughness of spider silk results from cooperation between structures and effective "self-healing" mechanisms over a range of length scales during deformation. At the molecular level, simulations have revealed the importance of β-sheet dimensions and confinement to produce tough as well as strong crystals: β-strands with four or fewer hydrogen bonds are predominantly loaded in uniform shear, allowing cooperative rupture of bonds that can reform at adjacent sites to create a stick-slip mechanism.[23,39–42] Protein mechanics are further modulated by the selective binding of water to proteins, which provides a mechanism for energy dissipation, restructuring, and self-repair.[35] The pseudoamorphous domains, particularly in Spidroin I, have been shown to restructure into β-sheets under applied loading, further acting to maintain the number of hydrogen bonds and provide energy dissipation over large deformations prior to the irreversible rupture of covalent bonds.[43] Combined molecular dynamics and finite element modeling at this level have allowed the decoupling of crystalline and pseudoamorphous domains to reveal viscous and friction effects that control bulk viscoelasticity.[44]

Fibril-level morphology provides further performance enhancements. Confinement in thin fibrils (∅20–80 nm) forces homogeneous deformation and cooperation between β-sheets to maximize resistance to failure.[45] The globular rather than cylindrical morphology of fibrils enhances stress sharing between fibrils and provides a stick-slip mechanism to provide local stress relief and energy dissipation to homogenize stress over the fiber, and resist the formation of fracture clusters.[22,46]

Recent mechanical models, e.g., Ref. 47, have built on these discoveries, attempting to combine the effects of different hierarchical mechanisms to examine their interactions and contributions to the bulk mechanical behavior of the spider silk fiber. Although these hierarchical models require further development to encompass the sophistication of many of the toughening mechanisms, they have provided insight into species differences and the effects spinning conditions on mechanics. Such models (including coarse-graining approaches[45]) are likely to play an important role in synthetic material design, complementing molecular dynamics approaches.[48] The improved understanding of structure–function relationships over the last five years is likely to allow more rational and quantitative design to produce tunable, high-performance synthetic silks.[8,49]

III. NATURAL SPIDER SILK PRODUCTION

Spider silks are produced in vivo through a combination of chemistry to produce a liquid crystalline "aquamelt" spinning dope[50] and a series of predominantly mechanical steps to induce restructuring, alignment, and phase separation processes during spinning.[3] Successful synthesis of a spider-like silk will require both the dope material and spinning mechanics to be reproduced.[51]

A. Genes and proteins

Despite maintained interest in spider silk proteins, particularly the major ampullate spidroins (MaSp1 and MaSp2), the full-length sequences of the genes encoding them have only recently become available. Sequence determination has been hampered by the sheer enormity of the proteins, which have estimated size ranges of 200–350 kDa,[52] with large interspecies size variations due to their repetitive sequence motifs.[53,54] The large number of short repeat regions encompassed within the proteins and their genes represents a formidable challenge for sequencing, precluding standard polymerase chain reaction (PCR) and primer walking strategies,[55] and hampering bioinformatics contig assembly.

Sequencing of a genomic library of the black widow spider, Latrodectus hesperus (Theridiidae), revealed that the MaSp1 gene is composed of a single exon with 9390 bp encoding 3129 amino acids. Similarly, MaSp2 contained an 11,340 bp exon encoding 3779 amino acids.[55] Analysis of the wasp spider Argiope bruennichi[56] confirmed that the sizes of MaSp1 and MaSp2 expressed mRNA are ~9 and ~10 kb, respectively. However, the recent publication of the full genome for the African social velvet spider, Stegodyphus mimosarum, reported 10 copies of the major ampullate spidroin genes, with heterogeneous repeats, exon–intron structures, and gene product sizes significantly less than 9–10 kb.[57] The detection of additional heterogeneity using next-generation deep sequencing is not surprising. The earlier studies relied on bacterial clone libraries, which may not contain every gene copy. However, the lack of consistency in detecting a single exon 9–10 kb gene product is unexpected and is likely to reveal new insights about the heterogeneity of spider evolution.

The highly ordered MaSp1 protein consists of poly-alanine (poly-A) and GGX motif repeats and the less-ordered MaSp2 consists of poly-A and GPGXX motif repeats (where P is proline and X is any amino acid). The crystalline regions of spider silk consist of 90% alanine, whereas glycine accounts for 70% of the residues in the disordered regions.[28] Together, these repetitive regions and their distributions are largely responsible for mechanical performance as described above. Interestingly, the repetitive region of MaSp1 and MaSp2 proteins varies considerably between and within species, while the N- and C-terminal regions are highly conserved.[58]

The high conservation of the nonrepeat regions among all spider species[58,59] indicates that, while not directly responsible for material properties, they are likely to be of high importance for synthesis. The charge distribution of the N-terminal domain provides a module that allows the intrinsic pH gradient of spider silk glands to regulate silk formation.[60] Salt concentration also affects the structure and stability of the N-terminal domain, providing a further mechanism to prevent premature fiber formation during storage.[61] The structural state of the C-terminal domain is essential for controlled switching between the storage and assembly forms of silk proteins.[62] This domain is indifferent to pH,[60] and has a role in the alignment of secondary structural features formed by the repetitive regions of spider silk proteins, and affects silk formation by ordering the assembly of repetitive segments into fibers.[62] These domains, and the environment that modulates them, are critical to synthesis and could be key to overcoming solubility problems in synthetic silk production.

Several groups have begun studying the greater environment of constituents involved in silk production within the gland, and the final entities embedded in the silk. For instance, proteomic analysis has identified more than 100 other proteins present in the silk, which may provide further insight into the material and its processing.[57] Additionally, the analysis of RNA transcripts within silk glands has indicated a host of potential proteins that may be involved in silk biogenesis.[63,64] In particular, Clarke et al. elegantly determined 647 silk-gland specific transcripts for Latrodectus hesperus (western black widow) by removing transcripts also found in other spider tissues (venom and cephalothorax). Transcripts can be broadly categorized according to function and ranked according to the number of transcripts associated with that category. Emerging major categories include translation and biogenesis, post-translational

modifications, signal transduction, protein degradation, inhibition of protein degradation, and oxidation–reduction (among many others).[63,64] Additionally, a large number of transcripts remain unassigned.[63,64] At present, all transcriptome generation has been performed on mixtures of silk glands, giving a broad overview of "silkomics" rather than specific insights into the molecular constituents of individual glands. Further deep-sequencing, transcriptome, and proteomic studies for additional spider families and individual glands will help reveal the true nature and number of MaSp genes, gene transcripts, and proteins involved in natural spider silk production and may provide interesting insights that can be used to improve synthesis.

B. Spinning process

Spider silk is produced by silk glands housed within the abdomen. Depending upon the species, a spider may have up to seven different types of silk glands, many of which appear at least in duplicate, such as the major ampullate, minor ampullate, and flagelliform glands.[65] There are six tubuliform glands (also known as cylindriform or cylindrical glands) and many small aciniform glands that produce the silk used by female spiders to wrap and protect their eggs. The four aggregate glands produce an aqueous glue-like protein for coating prey-capture silk strands within the web.[65,66]

The major ampullate gland anatomy can be divided into three main sections: the tail, the sac, and the processing duct (Fig. 1). In the tail and proximal sac, the main silk proteins (Sp1 and Sp2) are secreted from tall columnar epithelial cells, forming spherical droplets, which are stored as a liquid crystalline dope, in the ampoule-like sac section.[67] Within the sac, the conformation of the highly concentrated spidroins has been described as "molten globular", very different to that observed in the processed fiber,[68] which is likely to aid flow and storage. Cells lining the distal sac are thought to secrete coat proteins, most likely glycoproteins, to modulate water interactions[3] necessary for efficient spinning.[50]

During spinning, the liquid crystalline silk flows into the duct, which is responsible for the intense processing and ordering of proteins to transform the silk dope into the fibers that we recognize as spider silk. The major ampullate duct is folded three times in a gradually tapering structure, making it five times longer than would be necessary to simply attach the sac to the spinneret. The underlying mechanisms of restructuring and assembly within the duct are still being investigated,[68–71] however, the processing of silk proteins is known to include a decrease in pH, ion exchange, dehydration, elongation, and alignment.[72–74] Vollrath and Knight[3] suggest that the low, constant stresses in the long, hyperbolic duct, and the resultant formation of complex alignment patterns of molecules, suppresses the formation of defects during flow and restructuring in the early phases of spinning. Also critical is the internal drawdown within the third limb of the duct,[3,73] at which point the proteins aggregate, align, and crystallize, with high stresses and increased acidity inducing phase separation to solidify the fiber.

The duct attaches to one of three pairs of spinnerets, which can be seen on the ventral side of the abdomen, distal to the cephalothorax (Fig. 2). Here, a second drawdown occurs to form the final fiber. The spider has neuromuscular control over the width of individual spigots and therefore has control over the width of individual silk strands and the friction applied during the final spinning step.[75,76]

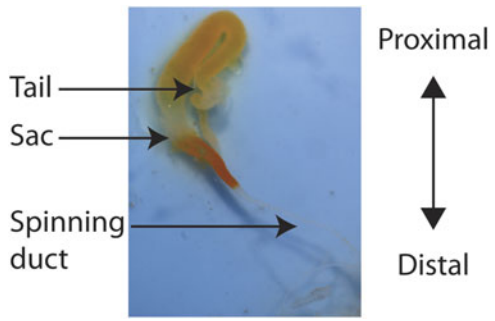

FIG. 1. The major ampullate gland. A silk gland dissected from a golden orb-weaving spider approximately 20 mm in body length showing the three anatomical regions.

IV. SYNTHETIC SPIDER SILKS

Unlike silkworm silk, various challenges preclude spider silk from easily being harvested. Spiders cannot be kept in close proximity to each other due to their territorial and cannibalistic behavior and cannot therefore be effectively farmed. Harvesting natural silk would also be economically prohibitive due to difficulties in collecting the spun material. This was illustrated by the recent production of a single spider silk cape,[77] made from silk collected from the golden orb weaving spider Nephila madagascariensis. With each spider producing 40 m of silk, which was forcibly reeled, it took more than 100 people approximately three years to complete and approximately 1.2 million spiders to produce. For any commercial application, it is therefore critical to produce the material synthetically.

A. Making synthetic silk

Heterologous silk protein studies have been conducted using a range of organisms including bacteria,[78] tobacco plants,[79] yeast,[80] silkworms,[81] goats,[82] and mammalian cells[83] (for an in-depth review, see Refs. 9 and 52). The field has advanced substantially in recent years,[9] yet the success of synthetic silk development has been highly variable with no study demonstrating a synthetic fiber that reaches the property and processing benchmarks set by natural spider silks. This could be due to a range of

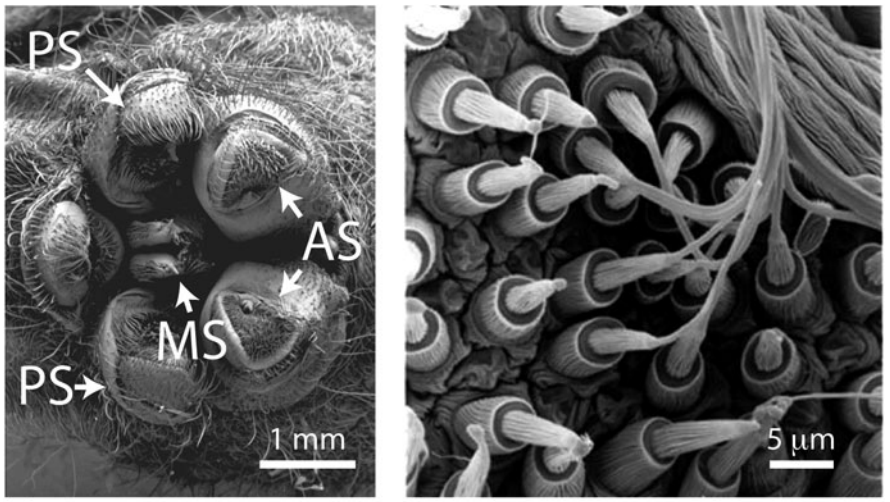

FIG. 2. Spinnerets and spigots of an orb-weaving spider. Electron microscopy of the (a) anterior lateral (AS), posterior median (MS), and posterior lateral (PS) spinnerets (photo by Sue Lindsay © Australian Museum) and (b) individual spigots on the surface of the spinneret (Photo by © Tina M. Weatherby).

factors including but not confined to limitations on the size of the gene inserted into the organism, limitations on the expression system of the recombinant organism, and a limited understanding of, and ability to replicate, the spider's natural spinning process.[3]

In addition to the lack of gene sequence availability, synthetic expression of any protein of 200–350 kDa presents several technical challenges. Recombinant expression of large proteins traditionally bestows a productivity cost, such as longer production times and reduced yields, both of which are undesirable for commercial-scale manufacturing. Thus, most researchers have focused on expression of gene portions, particularly the characteristic repeat regions.[79,83,84] The effect of the N- and C-terminal conserved portions of the proteins in terms of synthetic silk properties has only been partially investigated.

B. Silk gene expression

Some degree of success has been achieved in all of the above-mentioned organisms, but differences in codon usage have caused inefficient translations, requiring synthetic codon-optimized constructs to be developed. Additionally, since the genes are guanine and cytosine-rich, depletion of the tRNA pools often occurs before obtaining adequate protein yields.[52] High molecular weight expression, matching that of native silks, appears critical in obtaining high strength synthetic silk. This was demonstrated by Xia et al.[85] who produced a range of molecular weight silks in Escherichia coli (E. coli) and found a clear dependence of mechanical properties on molecular weight (see later).[85] Interestingly, bacteria with enriched tRNA populations express MaSp proteins with high efficiency, resulting in a reasonable yield for proteins up to native size (285 kDa).[85]

E. coli has been the system of choice for recombinant production of spider silk dope, however, low protein yields and poor solubility are hallmarks of E. coli expression.[86] Additionally, unwanted recombination events can lead to shortening of the repetitive genes.[52] One method to improve protein yield and purity is to secrete the protein into the extracellular media, which bypasses the intrinsic production limit of the total amount of protein that can accumulate within a cell. With Gram-negative organisms, such as E. coli, secretion requires crossing of the inner and outer cell membrane, which is a difficult process. Expression in a Gram-positive organism, Salmonella typhimurium (SPI-1 T3SS), has been attempted, where secretion is aided by needle-like structures that cross the membranes.[87,88] However, the degree of secretion was dependent on the length of the recombinant silk protein[87,88] and thus is not likely to be suitable for native size protein expression. Secretion is more likely to be successful using yeast or baculovirus expression systems, which have previously been demonstrated to process high molecular weight proteins.[52,89] Both baculovirus and yeast have been used to express silk constructs, but none have yielded similar strengths to that reported for E. coli above. In each of these systems, however, only smaller constructs were analyzed.

Other hosts such as plants[90] and mammalian cells[83,91] have also been demonstrated with silk constructs, yet these methods are also plagued with low expression levels and limitations in molecular weight.[52] Recent work using transgenic expression of ELPylated spider silk

proteins in tobacco, however, appears to have overcome the problem of low molecular weight expression.[92] After purifying the fusion proteins by inverse transition cycling and crosslinking with transglutaminase, high molecular weight protein films could be produced.

Reports on transgenic animals such as mice[93] or goats[94] to produce silk offer an intriguing future for silk farming. The production of transgenic animals is an extremely complex and time-consuming process, and hence this system would not be useful for general expression studies to probe structure–function relationships. However, for final-product manufacture, they may offer advantages since high molecular weight proteins can be obtained with high protein yield, if the separation of silk from milk caseins can be industrially performed.[52] While these methods have been reported for silk production, the resulting materials have not replicated the properties of natural spider silks. Beyond mammalian expression, the use of transgenic silk worms[81,95] is particularly interesting and would enable direct spinning of high molecular weight fibers (see below).

C. Biomimetic spinning strategies

Despite the very strong progress in protein expression to create a potentially viable spinning dope, little progress has been reported in the physical reproduction of the natural spinning process. For applications such as coatings, optics, non load-bearing scaffolds, wound dressing, and drug delivery systems, current spinning and deposition technology produces useful materials.[96,97] The quest to recreate the spinning conditions that would allow us to match, or even exceed, the properties of native spider silks remains in its infancy and is largely in the domain of intellectual property rather than the scientific literature.[98,99]

A promising approach to fiber synthesis is emerging from the field of microfluidics,[100] which provides a tunable fabrication process that allows for customizable fiber formation. Recently, microfluidic strategies have been used to explore biomimetic spinning on microchips. Simplifying the geometry of a silkworm spinning duct to an exponential function, researchers produced artificial silks tougher than degummed silk using a dry spinning method.[101] Mimicry of the more extended, hyperbolic-to-linear[102] geometry of the spider spinning duct has not yet been attempted, but is likely to provide further advances in performance. Microfluidic devices have also been created to allow the laminar flow of a mobile phase in parallel with the spidroin solution.[103] When a water-insoluble liquid, such as oil, is chosen as a mobile phase, the spidroin self-assembly takes place at the created interface, which moves with the flow. The amphiphilic spidroins accumulate at the interface, imitating water removal from the formed fiber. Although this work is preliminary in nature, these advances also provide a platform to explore the spinning process, allowing further understanding and optimization. Krishnaji et al.,[48] for example, have used a range of experimental and computational approaches to understand the relationship between structure and function in recombinant silk-like block copolymers that were spun with a tunable microfluidic approach,[100] yet did not report the effects of spinning conditions. A combined investigation of processing, design, structure, and function will substantially advance the field.

Focusing on the extrusion of the silk protein solution will be key for the next generation of engineered fibers, as it could be that the properties of the end product are more sensitive to the processing and extrusion of the dope solution compared to the protein sequence. Insensitivity of mechanical properties to protein sequence has previously been observed in bioinspired silks.[94] Innovations will require a better understanding of in vivo dope synthesis, rheology,[104,105] and chemistry[106] to optimize hierarchical structuring of the fibers.

D. Overcoming the mechanical performance deficit

A variety of approaches to optimize mechanical performance of synthetic silks are being explored. One challenge lies in both defining and synthesizing a "true" synthetic spider silk, as opposed to a generic silk-like material or simply a protein-based polymer, since all three classes of materials can have important structural (and functional) similarities.[27] The distinction is the subject of quite some philosophical scrutiny.[51] Here we discuss primarily biosynthetic recombinant spider silks, which are produced by suitable hosts and then purified and processed into fibers via extrusion of a dope solution,[78] and regenerated natural silks, in which natural silk proteins are extracted, chemically treated, and extruded.[72]

Fibers formed from regenerated natural silks have generally exhibited mechanical performance that compares unfavorably with natural spider silk, regardless of whether the regenerated silk was formed from aqueous solution[107] or from organic solvents.[108] Similar performance has been reported for recombinant silk. Producing silk from mammalian cells, Lazaris et al.[83] detailed the complexity and subtlety of the extrusion process and described the dependence of the fiber diameter, the birefringence (an indicator of crystal ordering within the fiber), and the silks' mechanical properties on processing parameters. Sensitivity to processing has also been noted in regenerated natural spider silk proteins,[108] though clear guidelines and mechanical optimization strategies have not been reported.

Molecular weight appears to be strongly linked to mechanical properties: the best mechanical performance has been observed in silks expressed with large proteins. The previously discussed innovations in metabolic

engineering of E. coli have allowed for the expression of spider silk proteins with controlled molecular weight from ~55 to ~285 kDa; the upper value is in the range of natural spider silk proteins.[85] The highest molecular weight silks from this study exhibited breaking strain and tenacity comparable to natural N. clavipes dragline silk, and a Young's modulus that exceeded that of the natural product (21 ± 4 GPa compared to 11–14 GPa, respectively).

Currently, the most promising method for synthesizing silk fibers with high molecular weight proteins is to use a host that is inherently capable of secreting large proteins, such as B. mori.[109] The early results in this area are encouraging: hybrid recombinant silks incorporating spider silk sequences produced by B. mori could be expressed with reasonable yields.[81] Fibers were found to be as tough as dragline spider silk, but exhibited a strength intermediate between B. mori and dragline spider silk.[81] B. mori, as a silk spinning insect, is therefore an ideal test bed to optimize sequences for commercial applications and to delineate the roles of protein sequence and processing in the properties of synthetic fibers to allow quantitative comparisons between research groups. The very different flow characteristics of spider and silkworm silk production,[110] however, suggest that this approach may be limited with respect to creating fully biomimetic spider silks.

A recent paper by Porter et al. suggests that some of the material properties of spider silk may simply be inherent to its small fiber diameter (<5 μm).[111] In this work, a meta-analysis was used to generate a universal curve for polymer fiber strength, showing that in a Griffth-like analysis,[77] a variety of polymer materials show similar fracture strength behavior that depends on fiber diameter (Fig. 3).[111] Diameter-dependence of mechanical properties had previously been demonstrated for fibers formed from another protein-based material, the recombinant Drosophila melanogaster transcription factor Ultrabithorax,[112] as well as for other materials like glass,[77] polyethylene,[113,114] polydiacetylene,[115] and other polymers[116] and composites.[117–119] Simulations using a coarse-grained mesoscopic computational model for spider silk fibers revealed similar behavior, showing that failure stresses and strains increased with decreasing fiber diameter, finally reaching the limit of a defect-free fiber at a diameter of 50 ± 30 nm.[45] Porter et al. note that toughness is dependent on the internal structure of spider silk, in particular its hydrogen bonds, which are critical for its high cohesive energy density.[111] Thus we arrive at a recipe of sorts for engineered silks: minimize diameter

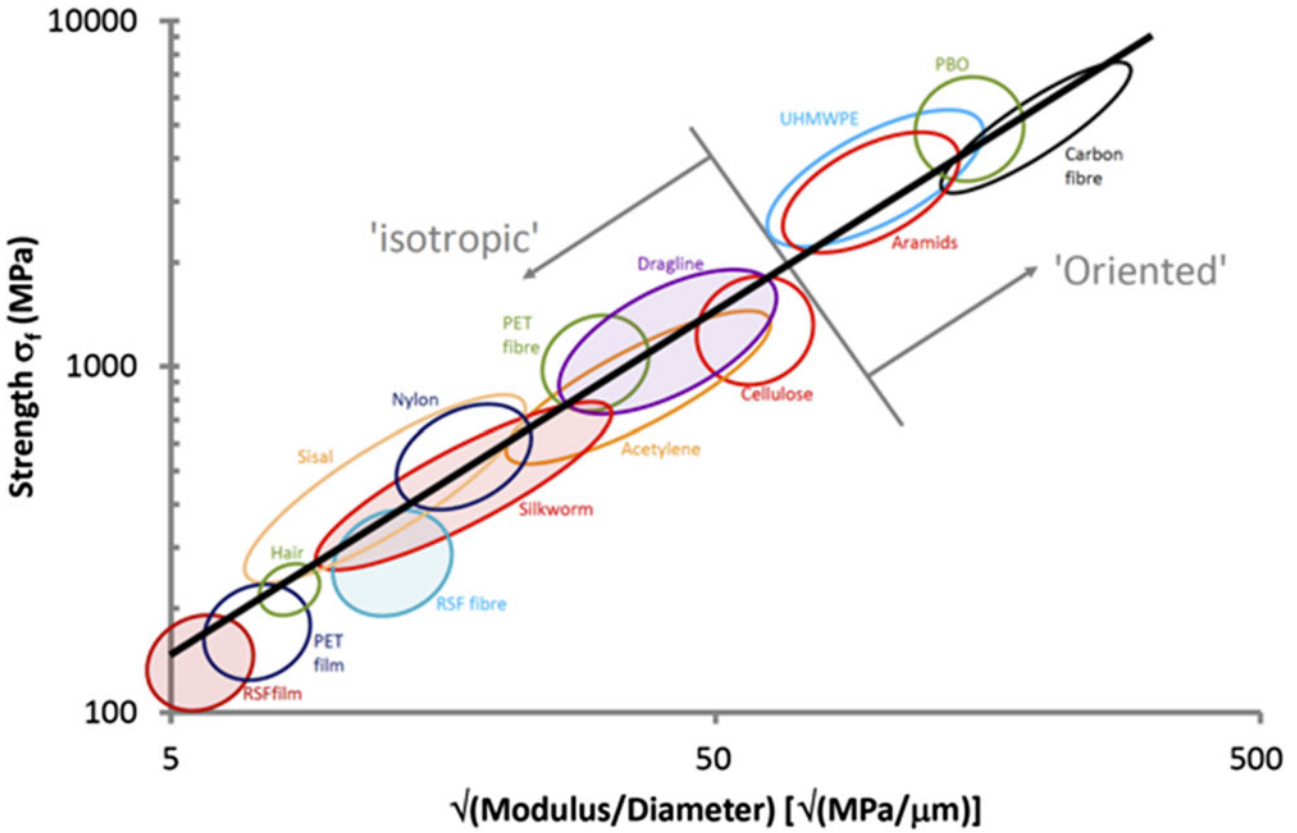

FIG. 3. Curve showing that the generalized fracture strength behavior of polymer fiber materials depends on the fiber diameter. A partial derivation of the relationship and references for the plotted values can be found in Ref. 111. Figure is reproduced with permission from Wiley.[111]

while increasing the availability of structural interactions in the dope for optimized strength and toughness. This work also draws attention to the importance of carefully reporting all physical data, especially fiber diameter, for fibers subjected to mechanical testing.

V. APPLICATIONS AND POTENTIAL OF SYNTHETIC SPIDER-LIKE SILKS

Natural silk fibers have long been used in medical applications, in particular as sutures,[120] exploiting their strength as well as their short-term biocompatibility. Several studies have examined the biocompatibility of spider silk and found it either does not provoke an immune response, or will only elicit a very minor immune response.[121,122] Inflammatory reactions to natural silks in medical studies have been associated with the presence of the glue-like protein sericin on the sheath of the fibers and are greatly reduced in fibers where the sericin has been stripped.[123] Processed silks avoid the sericin problem and have been demonstrated to be biocompatible both in vivo and in vitro,[124] although some outstanding questions remain about their long-term biocompatibility, especially during breakdown of the polymers.

Engineered silks, through their diversity of shape and structures, have found employment in a wide range of applications that extend well beyond suturing. Much of this work has been done using silk proteins obtained from B. mori (silkworms) rather than from spiders, for pragmatic reasons related to the ease of producing large quantities of the silk. We expect the same applications to be available to spider-like silks. B. mori silk-derived protein (fibroin) has been incorporated into a range of composite materials, including polymer/silk composites and inorganic/silk composites that exhibit a variety of useful properties.[125] Regenerated fibroin has been used in a wealth of applications due to its ease of processing into a variety of shapes (Fig. 4),[126] meeting with particular success as biomaterial scaffoldings,[127,128] and as a carrier for targeted drug delivery,[129] where biodegradation in vivo is a required trait for the delivery microcapsules. Silk proteins have also been investigated as antimicrobial peptide carriers with a view for the development of a controlled-release drug delivery system.[130,131]

Silk fibroin has also formed the basis for a number of forward-thinking applications in sensing. A proof-of-principle demonstration showed that fibroin can form the foundation for implantable electronics, creating the type of biotic/abiotic interface necessary for long-term, active medical devices and sensors.[132] Fibroin has also been used in a number of optical applications, which we briefly describe here. When formed into photonic crystals with an inverse opal structure, fibroin films take on an optical property known as structural color; this same phenomenon is the origin of the brilliant reflected coloration, for example, of some insects and beetles.[132] The structural color of the inverse opals is measurable even when the films are implanted below 5 mm of

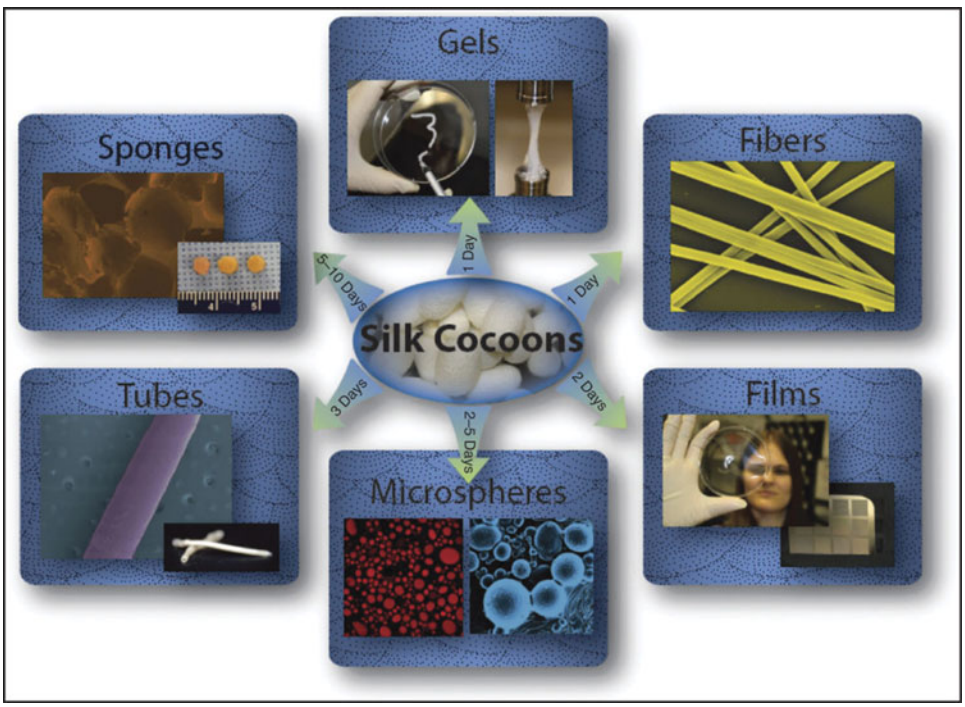

FIG. 4. Time for processing extracted silk fibroin solution into various structural motifs. Figure reproduced with permission from Nature Publishing Group.[126]

biological tissue, making these films excellent candidates for implantable sensing applications.[133,134] Furthermore, when the inverse opals are doped with gold nanoparticles, the optical properties of the film enhance laser-induced heating of the nanoparticles, demonstrating their potential for targeted laser-heating therapy. Fibroin inverse opals can be used as optical humidity sensors, as the humidity-induced structural changes of the protein structure translates to a change in the optical properties of the photonic crystal (Fig. 5).[135] Photonic properties can also be engineered into fibroin films by patterning them as gratings,[136] which then exhibit a specific spectral response that can be modified by submerging them in fluid,[137] or, in the case of functionalized gratings, by exposing to targeted gases.[138]

VI. CONCLUSIONS AND OUTLOOK

The development of new materials enables expansion of creativity by designers, engineers, architects, and scientists. The interest in spider silk goes far beyond its strength and toughness as rarely does a single fiber possess so many intriguing and diverse qualities. The remarkable results generated in recent years demonstrated just how relevant this ancient material can be to modern materials science. Spider silk expression studies have made major advances: the successful expression of native-size proteins in synthetic constructs has vastly improved the performance of final materials. There remains room for improvement in expression systems to readily express the protein for purification, with suitable conformation and at a sufficiently high concentration for efficient spinning, yet it appears that spinning technology is a major and under-explored obstacle to synthesizing a truly biomimetic material.

In addition to advances in synthesis, the route to controlling and fully emulating the structure and function of biological spider silks will require further improvements in our fundamental understanding of the material. As structure–function relationships are established, new areas of potential application can be explored. One example is the conflicting recent works suggesting that N. clavipes dragline silk may[139] or may not[140] possess a remarkably high thermal conductivity, on par with copper. Atomistic simulations suggest that, in particular, the β-sheets have an unexpectedly high thermal conductivity related to their inter-strand hydrogen bonding.[141] Fully understanding, and emulating, these attributes remains an ongoing pursuit, as research continues to reveal the novel, potentially useful, properties of natural silks beyond their well-known mechanical performance.[142]

In the quest of a spider-like silk fiber, it is important to note that reproducing the precise mechanical properties of native spider silk may not necessarily be useful. First, spider silk has evolved as a single-use material, which is perhaps its greatest weakness as a commercial fiber.[143] Used as a load bearing material, spider silk would be expected to withstand months to years of repeated stresses. It would therefore need to operate in its elastic region (below 2% strain), where its high strength and toughness cannot manifest. Although some post-yield deformation can be recovered by immersion in water for up to 30 min,[35] which may be useful under extreme, intermittent conditions, the post-yield characteristics are of little value in normal function. Second, the structural interactions with water that provide spider silk with its unique properties under ambient conditions will shrink and weaken a wet fiber. This phenomenon, known as supercontraction,[144,145] acts to maintain web tension in natural silks and can be harnessed as an attribute in some applications.[135] The severely degraded mechanical properties of a wet fiber, however, may further limit applications in biomedicine and other potentially humid environments. By tuning structure to suit the physical and environmental demands of an application, it is likely that for real-world applications, synthetic silks will outperform their natural counterparts even without matching

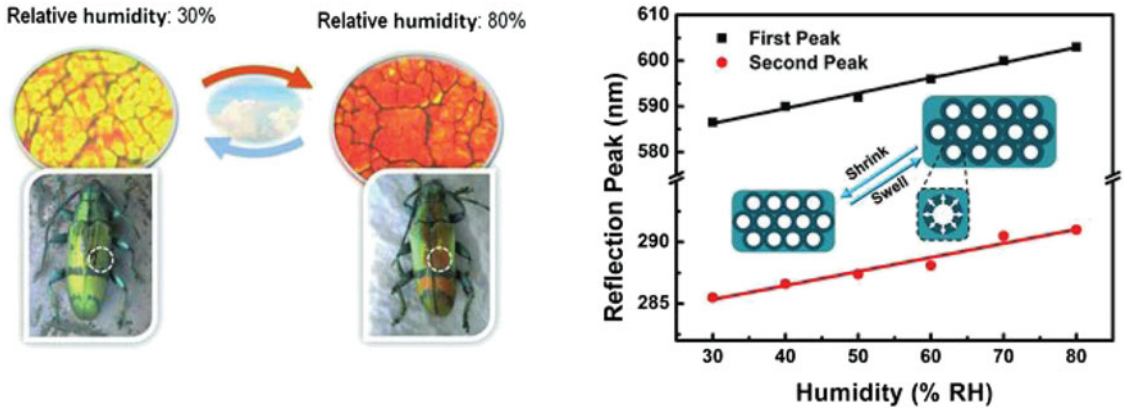

FIG. 5. Biomimetic structural color as a humidity sensor. Similar to certain beetles (left), fibroin inverse opals exhibit humidity-dependent optical properties (right). Figure reproduced with permission from Wiley.[135]

the benchmark set by their stress–strain characteristics in the laboratory. In the authors' opinion, the requirements of an application, rather than the reproduction of an idealized laboratory-based test, should be the benchmark and main aim of spider-like silk synthesis.

Spider silks possess a veritable cornucopia of useful properties, which provide opportunities for application-targeted engineered materials while at the same time exhibiting a rich phenomenology. The outlook for these tailored engineered biomaterials is very good: recent progress shows that in general, selected aspects of spider silk can be mimicked with a reasonable amount of success, and that engineered silks can respond admirably to the materials demands of specific applications.

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