Spider silk as a blueprint for greener materials: a review

Thierry Lefèvre* and Michèle Auger

Department of Chemistry, Regroupement québécois de recherche sur la fonction, l'ingénierie et les applications des protéines (PROTEO), Centre de recherche sur les matériaux avancés (CERMA), Centre québécois sur les matériaux fonctionnels (CQMF), Université Laval, Québec, QC, Canada, G1V 0A6

* Corresponding author: thierry.lefevre@chm.ulaval.ca

Abstract

Spider silk exhibits remarkable properties, especially its well-known tensile performances. They rely on a complex nanostructured hierarchical organization that researches progressively elucidate. Spider silk encompasses a vast range of fibers that exhibit diverse and captivating physical and biological characteristics. The full understanding of the relation between structure and properties may lead in the future to the design of a variety of high-performance, tailored materials and devices. Reknown for being produced in mild and begnin conditions, this outstanding biological material constitutes one the more representative example of biomimetism. In addition, silk's structure is produced with limited means, i.e. low energy and relatively simple renewable constituents (silk proteins). Then, if successfully controlled and adequately transposed in biomaterials, some properties of natural silk could lead to innovative green materials that may contribute to reduce the ecological footprint of societies. In fact, striking recent advanced applications made with *B. mori* silk suggest that spider silk-based materials may lead to advanced resistant and functional materials, then becoming among the most promising subject of study in material science. However, several challenges have to be overcome, especially our ability to produce native-like silk, to control biomaterials' structure and properties, and to minimize their ecological footprint. This paper reviews the characteristics of spider silk that make it so attractive and that may (or may not) contribute to reduce ecological footprint of materials and the challenges in producing innovative spider silk-based materials. First, from a biomimetic perspective, the structure and models that explain the tensile resistance of natural silk are presented, followed by the state of knowledge regarding natural silk spinning process and synthetic production methods. Biocompatibility (biosafety and biofunctionality) as well as biodegradability issues are then addressed. Finally, examples of applications are reviewed. Features that may lead to the design of green materials are emphasized throughout.

Keywords

Spider silk; Hierarchical structure; Spinning process; Green material

1. Introduction

Spider silks, proteinaceous filaments secreted by specialized abdominal glands of Araneomorphae, form one of the main hallmarks of biomimetism.[1] Dragline silk plays this role for long because this thread is renowned for its startling mechanical properties: a combination of strength and extensibility that provides an incomparable toughness and outclasses any industrial material.[2-7] These performances make spider silk the subject of intense research and position it as a very attractive biomaterial for future applications.

Besides its impressive tensile property, dragline silk also exhibits intriguing torsional characteristics and a particular water-induced physical response called *supercontraction* that may find original applications in various fields of materials science. Spider silk seems also to exhibit a low toxicity and immunogenicity, a slow biodegradability, and seems suitable to cell adhesion and growth, thus making silk very attractive for biomedical needs. In addition, like other proteins, spider silk proteins, usually called *spidroins* (a contraction of *spider* and *fibroin*), may be processed under various colloidal and physical states such as films, capsules, gels, emulsions, foams, porous systems, non-woven fiber mats and, of course, fibers.[8] Therefore, many opportunities may be found to harness the advantageous qualities of spidroins. Biotechnology even promises the possibility of producing materials with customized properties by designing tailored, spidroin-inspired sequences.

In its general sense, silk refers to the wide family formed by proteinaceous fibers excreted by arthropods, especially spiders, acarids and insects (moths, ants, bees, wasps, lacewings, etc.).[9,10] Silk fibers spun by arthropods display a variety of properties and structures.[2,3,5,9,11] A famous example is given by the silk produced by the domestic mulberry silkworm *Bombyx mori* (*B. mori*) which allows building a protective cocoon during worm's metamorphosis and that has been used by Humans for millennia due to its large availability from farming. Because of this advantage, and despite the fact that it is less mechanically performant than spider silk, it is actually the center of very stimulating researches that benefit from a reasonable tensile strength among other properties, including relatively easy and reproducible processability as well as usage flexibility.[12-16]

Among silk-secreting animal orders, Araneae is peculiar in that it is the only one in which species produce different types of silk. Spiders that spin aerial orb webs (family Orbiculariae) for instance can spin up to seven threads for various biological purposes, especially food, reproduction or displacement. Several works have shown that besides the dragline, other types of silks produced by spiders also exhibit diversified mechanical abilities[2,5,17,18] and molecular structures.[19-22] In all cases, silk properties are dictated by structures, which to date appears complex and hierarchically organized. Structures are in turn determined by protein sequence and by the ensemble of molecular events that occur during the spinning process, in a finely-tuned biological and chemical environment. Since each type of silk is made of specific proteins, giving rise to specific molecular, nano- and micro-structural organizations as well as particular attributes, spider silk represents a rich and prolific area of research from which many lessons can be learned and that may lead to fruitful developments in materials science, with the possibility to manufacture tailored fibers or materials.

It is thus not surprising that spider silk is considered as one of the most promising material in the years to come for industrial applications in the area of fibers.[23] More generally, silk may be the source of plenty of potential attractive applications in the field of materials, including in optics and photonics,[12,24] electronics and optoelectronics,[24] cosmetics, micro-mechanical systems,[25] textiles, tissue regeneration (scaffold for development of cells for various tissues)[15,16] and drug delivery devices.[15,26-29] Moreover, the way it is processed in nature, i.e. in mild conditions of temperature and pressure and in a benign solvent (water), as well as its biocompatibility and biodegradability properties may suggest that the formation of future synthetic silk will bring about (reasonably) inoffensive and green fabrication processes. Finally, as a proteinous material, spidroin-based materials are biodegradable, can be valorized and can be produced from renewable resources.

Taken into account all characteristics of the natural threads, spider silk-inspired biomaterials may probably lead eventually to the production of industrial goods with a minimal ecological footprint.[30] And for some applications, they may advantageously substitute for fossil fuel-based polymers. To date, one of the main restrictions is that scientists still experience difficulties to reproduce production procedures as benign as natural spinning and to form biomaterials with equivalent tensile responses than the native fiber. A second one lies in the fact that the supply of spidroins at large scale is complex, thus making it actually prohibitive economically. Advances and successes achieved in the last decades however suggest that some of these pitfalls are likely to be solved in the future. Therefore, due to the numerous potential qualities and benefits they include, spidroin-based materials seem to represent some of the best candidates for future green applications.

In this article, we review in detail the exceptional features of natural spider silk and the potential benefits (or shortcomings) for synthetic biomaterials, especially in the objective of reducing the ecological footprint of spider silk-inspired materials. This review then focuses on the structural organization of native fibers, the natural and artificial spinning processes, as well as biodegradability and biocompatibility properties. Then, possible future applications of spidroin-based materials are described.

2. Spider silk structure determines its properties

2.1 Spider silk tensile properties

Spider silk, especially the dragline, is reknown for its tensile properties since they are superior to any industrially-manufactured materials,[2-7] even high-performance ones such as Kevlar or carbon fiber (Table 1). This is due to an exceptional combination of high strength (breaking stress) and extensibility (breaking strain), resulting in a superior toughness (the work required to break the fiber) (see the inset of Figure 2 for the definition of the tensile parameters). Only recent laboratory-scale materials such as single wall carbon nanotubes are tougher (Table 1). Dragline's mechanical resistance has motivated the majority of researches as they aim at understanding its structural origin and at designing equivalent synthtetic biomaterials.

Table 1. Comparison between spider silks fibers, other biological fibers and synthetic fibers.

Material class	**Materials**		**Young modulus (GPa)**	**Strength (GPa)**	**Strain (%)**	**Toughness (MJ/m^3)**	**Ref.**
Biological materials	Forcibly spun MA silk	*Nephila clavipes*	13.8	1.22	19	111	31
		Argiope trifasciata	9.2	1.14	24	115	
		Araneus gemmoides	8.6	1.41	27	164	
		Latrodectus hesperus	10.2	1.44	35	181	
		Caerostris darwini	11.5	1.65	68	354	
	Flag silk	*Nephila clavipes*	~ 0.001	0.14	517	27	17,32
		Argiope aurentia		0.39	385	65	
		Araneus gemmoides		0.50	362	80	
	Ac silk	*Argiope trifasciata*	10.4	1.05	40	230	17
	Cocoon silk (degummed)	*B. mori*	7	0.5-0.6	15-18	70-80	7,33
	Limpet teeth	*Patella vulgata*	120	3.00-6.50	4-8	n.d.	34
	Cellulose nanocrystal		100-220	7.50-7.70	n.d.	n.d.	
	Dragonfly tendon (resilin)		0.002	0.004	190	4	35
	Mussel byssus (*Mytilus californianus*)	Distal	0.87	0.075	109	45	
		Proximal	0.02	0.035	200	35	
Synthetic materials	Single wall carbon nanotube rope		320-1470	13-53	1.1-5.3	~ 600	36,37
	Woven single wall nanotube fiber		80	1.8	30	456	38
	Kevlar 49		130	3.6	2.7	50	7
	Carbon fiber		300	4	1.3	25	

As illustrated in Table 1 with orbicularian species, the tensile characteristics of dragline fibers exhibit large phylogenetic varations. Emblematic high-peformance dragline silks are in fact spun by modern orb-weaving spiders, while those spun by more ancestral spiders are weaker and less extensible[39] as mechanical properties evolved with ecological requirements. In Orbiculariae for example, mechanical traits of silk threads seem to have coevolved with change in feeding behavior (use of orb webs) to resist the impact of flying preys, which in turn requires the optimization of the capacity of orb webs to dissipate the kinetics energy received. Such a new function for silk has been in part accomplished by changes in spidroin primary structure.[39]

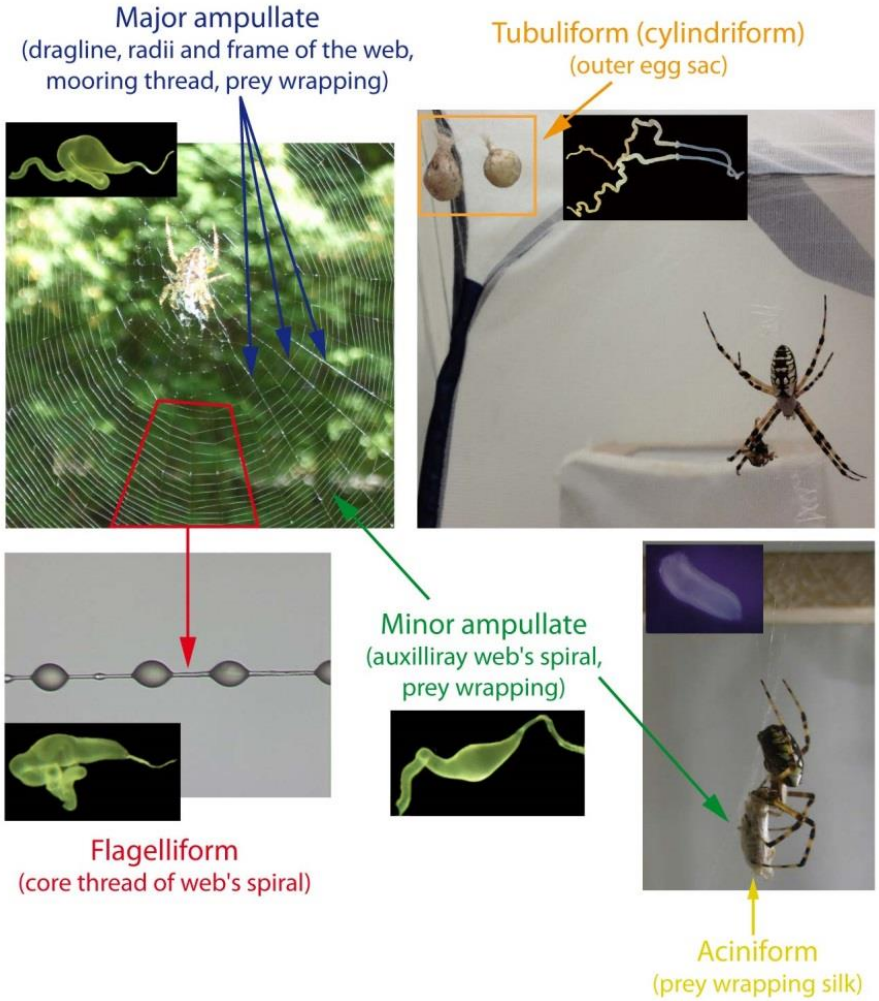

Figure 1. Illustration of the different types of silk spun by orb-weaving spiders, their biological function and the glands that synthesize and produce them.

Orbicularian spiders can spin seven types of silk that are named from their secreting glands.These specialized glands convert the spidroin dope solution into the solid fiber (Figure 1). The major ampullate (MA) glands for instance excrete the dragline or lifeline that is also used to make the radii and frame of

the web as well as the mooring thread. The flagelliform (Flag) silk (or viscid silk) constitutes the core thread of the web spiral. The tubulliform (Tub, also called cylindriform (Cyl)) silk is used to build the outer cocoon of the egg sac, the pyriform (Pyr) silk forms attachment disks for web and silk anchorage, and the aciniform (Ac) silk allows spiders to wrap their prey[40] and for somes spiders is used to weave decorations called stabilimentum.[41] Finally, the minor ampullate (MI) silk makes a temporary spiral but it is also found in the prey wrapping net.[20,42]

Each type of silk has one or several biological functions and are associated with specific mechanics. Tensile properties of other threads than the MA fiber have been the subject of few investigations although that would be very fruitfull to the design of new biomaterials. These threads are not all as strong as MA silk, although each one exhibits various combinations of strength and extensibility.[17] Flag silk for example has a quite lower breaking stress but is extremely extensible (Figure 2), so that like MA silk, it exhibits a high toughness.[5,32,43] Moreover, it also possesses a self-healing ability.[44] The aciniform silk is another example of intriguing material since it is a very thin filament that is tougher than MA silk for *Argiope argentata*[17] due to a slightly smaller rupture strength and a higher breaking strain. Such disparity is determined by particular structural organizations but they are less documented than for MA silk.

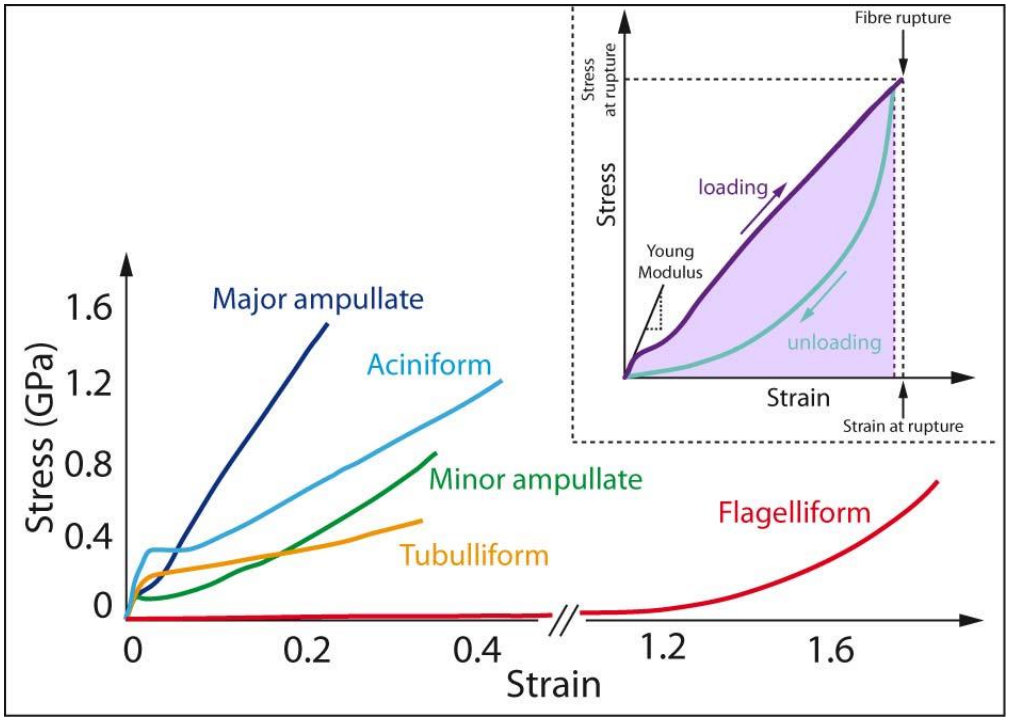

Figure 2. Tensile properties of silk. The diversity of mechanical properties of spider silk is illustrated by the strain-stress curves of different types of silk spun by the orb-weaving spider *Argiope argentata* (Adapted from Blackledge & Hayashi J Exp Biol 212 (2009) 1990). The inset shows the parameters used to characterise tensile properties. The purple area represents the work required to stretch the fiber (toughness) until a given strain. The area included between the loading and unloading traces represent the energy dissipated during the process of extension/relaxation.

The intrinsic mechanical propeties of the MA silk are fascinating from a fiber materials point of view but it is also relevant to understand to what extent they are adapted to biological functions. The MA silk has to resist to various constraints, such as the falling body weight of spider in the case of an emergency, wind forces that tend to pull out webs form the substrates on which they are attached, the impact of flying preys.

Silk mechanics have then major consequences on web resistance againt the impact of a flying prey. Web properties are overall determined by fiber tensile properties and web architecture.[45] The resistance of webs is particularly demanding since they are discrete in nature and have to be designed with a minimum of material and efforts to reduce the metabolic cost of their contruction. This is particularly challenging for orb webs as they are a monofilament planar network structures (Figure 1).

If fibers' tensile parameter values are essential for web mechanics, it is also important that fibers dissipate the kinetics energy of the prey. Fibers should then be viscoelatic rather than purely elastic, since in the latter case preys could be ejected back if the return of the web towards its equilibrium position was too energistic.[5] In this respect, MA and Flag silks exhibits a strong hysteresis upon a loading/unloading cycle (Figure 2) so that they can absorb nearly 65% of the input energy[7] that is disissipated by heat loss through viscoelastic processes.[5] It appears that radial threads provides the main contribution to orb web's energy dissipation.[46] Moreover, the non linear tensile response of the MA silk, especially its strain hardening behavior, seems to also be beneficial to web robustness, especially by restraining deformations to localized area.[47]

2.2 Spider silk proteins (spidroins)

Each type of silk thread is made of specific spidroins and exhibit a specific mechanical behavior which originate from their complex molecular, nano- and micro-structural organization, which in turn is determined by both protein sequence and various factors that influence the assembly during the spinning process. Thus, to be able to produce future performant and green biomaterials, it is of prime importance to acquire a detailed knowledge of these structural organizations and an advanced understanding of the relationships between spidroin sequence and fiber structure.

Spidroins are high molecular weight (typically 250-350 kDa) fibrous proteins whose sequence varies with the type of silk and with spider species. Nevertheless, spidroin sequences of spiders that belong to the same clade share close patterns for a given type of silk, giving rise to silk fibers with comparable mechanical properties. This is expected since a given type of silk fullfils the same biogical function.

The complete sequence of several spidroins has been determined. The canonical architecture of any spidroin consists of a highly repetitive (Rep) sequence segment flanked by N- and C-terminal (NT and CT) non repetitive (NR) domains (Figure 3). As an example, the Rep domain of the MA silk typically comprises a 30-40-long amino acid sequence that is repeated about a hundred of times, the NR segments being about 100 amino acids long. The NR terminal domains are highly conserved among spiders and types of silk, suggesting fundamental roles in silk assembly and structure, especially in spidroin storage

stability and spinning assembly (see below).[48-53] NR sequence regions have been found to be folded into α-helix bundles in solution[48,49] and are highly sensitive to the aqueous environment.

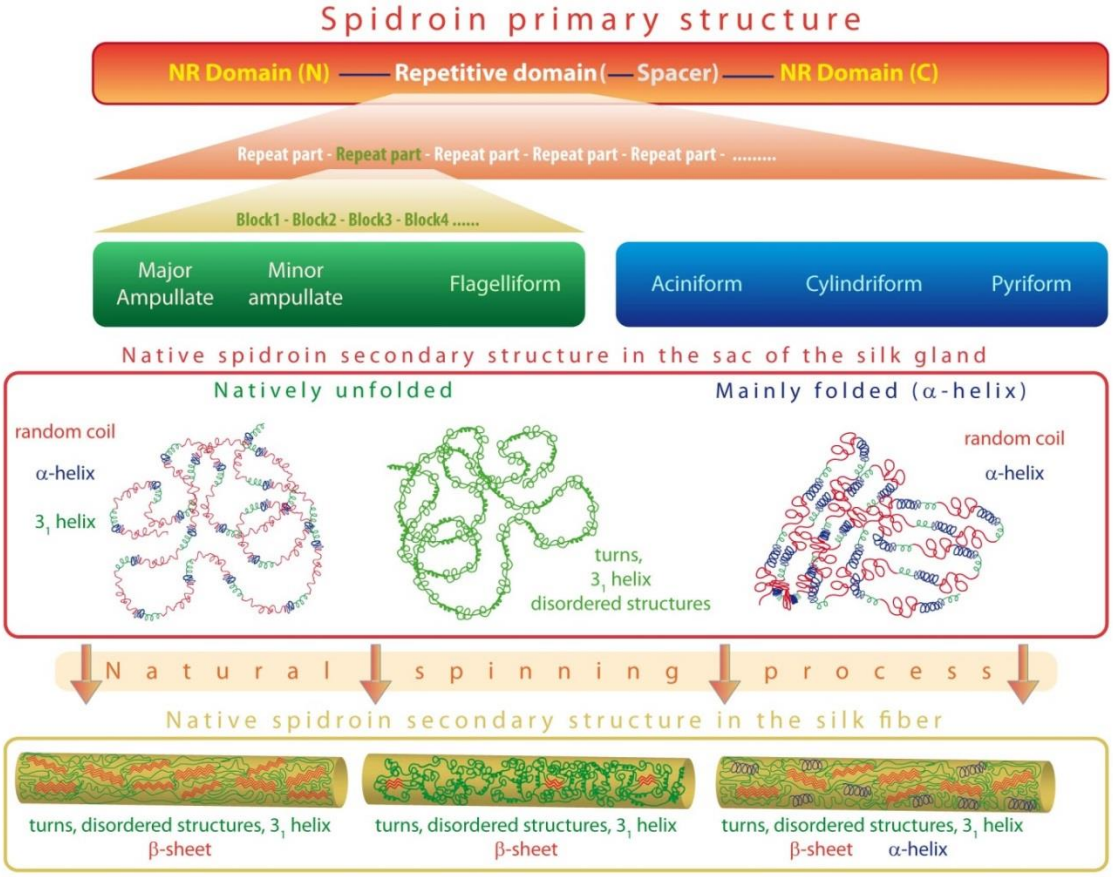

Figure 3. Canonical architecture of the spidroin sequences of the different types of silk of a typical orb-weaving spider. The schematic native secondary structures adopted by the proteins in the sac of the silk gland and after spinning in the fiber are also shown. Whereas spidroins are all composed of a repetitive domain, the repeat part may be, or not, decomposed of smaller modular motifs (blocks) such as polyalanine, GGX, GA or GPGXX. This distinction between spidroins containing or devoid of modular motifs seems to be related to two types of conformation in the dope solution: basically natively unfolded or mainly folded. It is however not related to distinct fiber structural families. Modified from Lefèvre et al. *J Mol Biol* **405** (2011) 238.

Spidroin primary structure is specific to spider species and to the type of silk. The different silk fibers can indeed be distinguished from the repetitive domain of their sequences. Some of them (MA, MI, Flag) can be decomposed into smaller, more or less hydrophobic, repetitive block motifs, whereas others (Ac, Tub, Pyr) do not exhibit such a pattern (Figure 3 and 4).[54-57] Motif repetitiveness of dragline spidroin sequence has increased with evolution and is associated with enhanced strength of the fiber.[39] The presence or absence of repetitiveness in spidroins secreted by orbweavers seems to be related to the type of secondary structures adopted in the dope solution (initial state): whereas former spidroins are essentially disordered and belong to the natively unfolded protein family, latter ones are mainly folded

into α-helices in solution (Figure 3), which questions the role of preliminary folding/unfolding on the efficiency of the spinning process.[21] Some spidroins (MI, Flag) also contain a (repeated) spacer region with an unspecific sequence and unclear role.

2.3 Relation between prmimary and secondary structures

A commonly admitted paradigm stipulates that repetitive block motifs of MA, MI and Flag silks adopt specific secondary structure in the fiber. Which secondary structure is associated with certain sequence motifs is however not completely understood. Moreover, the relationship between sequence and secondary structure remains unclear for spidroins that are devoid of internal iterated motifs. A better understanding of the structural and mechanical role played by the different sequences of spidroins would be very fruitful to the design of high-performance biomaterials.

MA spidroins contains short polyalanine runs (4-10 Ala residues) that form stiff and highly oriented[58,59] β-sheet nanocrystals dispersed within a soft amorphous matrix (Figure 3 and 4). The amorphous phase is composed of hydrogen-bonded polypeptide chain segments rich in glycine residues such as GGX motifs that adopt still ill-defined, but more disordered, secondary structures such as 3_1-helices, β-spirals, turns and/or random coils,[54,60-64] and that surprisingly seem to be only very slightly oriented.[62,65] A similar structural pattern is also observed for *B. mori* silk, although the crystallinity[66-71] and the proportion of β-sheets[62,65] are higher.

The appearance during evolution of the GGX motifs ~230 millions years ago is associated with fibers that have a higher breaking energy higher than Kevlar, although still less though than modern Orbiculariae MA silk.[39] Further increase in frequency of the GGX motifs in the sequence during Evolution is related to an increase in fiber tensile strength, extensibility, work to fracture and capacity to shrink (see below).[39]

Silk threads such as MI and MA silk can be made of one or two proteins. The MA fiber of orbweaving spiders is made of two spidroins called MaSpI and MaSpII (major ampullate spidroins I and II) that share the same primary structure pattern (Figure 4). They however differ in the details, in particular in the fact that MaSpII repeat sequence contains Pro residues whereas they are lacking in MaSpI. MaSpII is in fact a "novel" protein that appeared with the orbicularian clade. This evolutionary change is related to a higher extensibility and toughness of MA silk with regard to non orbweb spiders.[39,72] These property changes are mainly attributed to the proline residues of MaSpII that are incorporated into GPGXX motifs. Proline prevent the formation of regular secondary structures while GPGXX blocks are hypothesized to play the role of nanosprings, thus confering mobility to the polypeptide chains.[22,54,73,74]

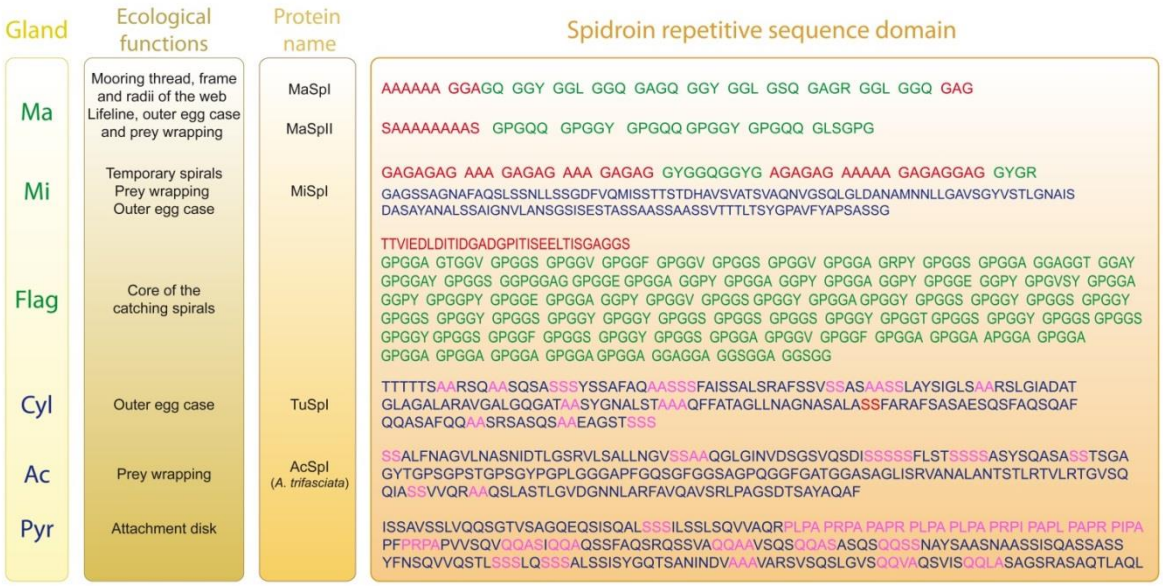

Figure 4. Repetitive sequence of the silks produced by a typical orb-weaving spider (*N. clavipes*). Red and green letters represent amino acids involved in β-sheet and disordered structures in the fiber, respectively. Blue and purple amino acids adopt an unknown secondary structure (purple ones represent irregular repetitive blocks). Sequences with the following IDs were derived from UniProt ((http://www.uniprot.org/): P19837 (MaSpI),[75] P46804 (MaSpII),[76] O17434 (MiSp1),[77] Q9NHW4 (Flag),[78] Q3BCG2 (Cyl),[79] and G0WJI5 (Pyr).[80] Since the sequence of the wrapping silk for *N. clavipes* is unknown, the sequence of the wrapping silk (AcSp1, Q64K55)[81] for a closely-related spider (*A. trifasciata*) is presented. Adapted from Lefèvre et al. *J Mol Biol* **405** (2011) 238.

Based on the secondary structures of the threads spun by *Nephila clavipes* spiders, three families of silks have been identified.[21] The first one is composed of threads that share the structural pattern of MA silk, including the MI[82-86] and Tub[83,85,87] silks. The cocoon silk of *B. mori* also shares the same structural pattern. The second family is formed by Flag silk, a fiber that experimentally showed almost no molecular orientation and disordered proteins,[20,88] consistently with its large extensibility. This feature mainly relies on the spidroin chemical structure dominated by GPGXX motifs (Figure 4) that would play the role of nanosprings as these motifs are supposed to form β-spirals.[54] Nevertheless, disordered conformations have also been proposed.[20,21,89,90]

It has been found that a particular region of the sequence of Flag spidroin, a 15-30 amino acid irregular segment called spacer (Figure 4), also forms β-sheets and exhibits a moderate orientation, in a similar way but in a quite smaller extent than MA silk (Figure 3).[88] The data suggest a role of this segment on the mechanical behavior of viscid silk.[88] Finally, the third structural family of orb-weaver fibers is composed of the aciniform and pyriform silks that are characterized by moderately oriented proteins in β-sheets and α-helices (Figure 3).[21] The contribution of α-helices, in addition to that of β-sheets and more disordered chains, may be essential to the large toughness of aciniform silk.

As can be seen, the β-sheet secondary structure appears as a ubiquitous structural element to produce tensile-performant silk fibers. This is consistent with the particular intrinsic properties of β-sheet structural elements. The content and level of orientation of β-sheets vary with the type of silk (Figure 5). These differences and the presence of other structural elements contribute to the modulation the properties of silk threads.

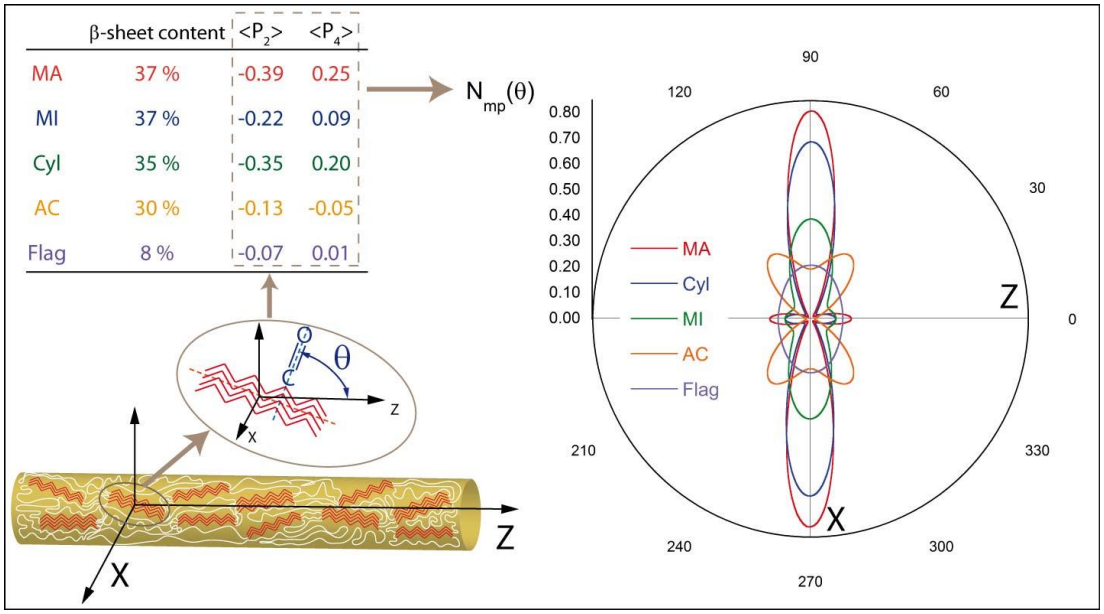

Figure 5. Level of orientation and content of β-sheets (in red) for different fibres spun by the spider *N. clavipes*, as determined by Raman spectromicroscopy. The left part shows the axis coordinate, schematics of the fibre and a particular β-sheet structural unit (β-sheets are represented in red, amorphous regions in white). The table shows the β-sheet content and the order parameters $\langle P_2 \rangle$ and $\langle P_4 \rangle$ that are measurable by Raman spectroscopy using the amide I band which mainly originates from the C=O stretching vibration. The right part shows the most probable orientation distribution function ($N_{mp}(\theta)$) of the β-sheets in polar representation as estimated from $\langle P_2 \rangle$ and $\langle P_4 \rangle$. The level of orientation is higher (the function $N_{mp}(\theta)$ is narrower) in the order MA > Cyl > MI > AC > Flag. All values are taken from Rousseau et al. *Biomacromolecules* **10** (2009) 2945 except for Flag silk ones that are taken from Lefèvre & Pézolet *Soft Matter* **8** (2012) 6350).

2.4 The structural organization of the MA silk fiber

A complete structural model that fully captures the outstanding mechanical performance of silk is still lacking, but several aspects are progressively elucidated. Most of the studies have concerned MA silk since it can be obtained in reasonable amount thanks to forced spinning (silk "milking"). This method consists in drawing silk artificially from the spinneret using a motorized speed-controlled reeling system while the animal remains awaken.

As seen above, and as illustrated by X-ray diffraction (Figure 6), silk is a semicrystalline material in which the β-sheets form nanocrystals. Such structure lead the pioneering model of Termonia[91] in which

the β-sheet crystals represent reinforcing cross-links embedded in a hydrogen-bonded rubberlike matrix exhibiting entropy elasticity (Figure 7). The orientation level and the crystallite size are important for the tensile breaking strength and yield stress.[92,93] Using a mean field theory, the main features of silk stress-strain curves have been reproduced at small and large strains.[18,94] The mechanical behavior could be captured using a single structural parameter, the fractions of ordered and disordered polypeptide chains, assuming that the disordered amorphous fraction undergoes rubber ↔ glassy transitions. Silk tensile behavior can then be rationalized by a balance between stiffness/strength on one hand and toughness/extensibility on the other hand, that are determined by elastic energy storage and energy dissipation, respectively.[18,94]

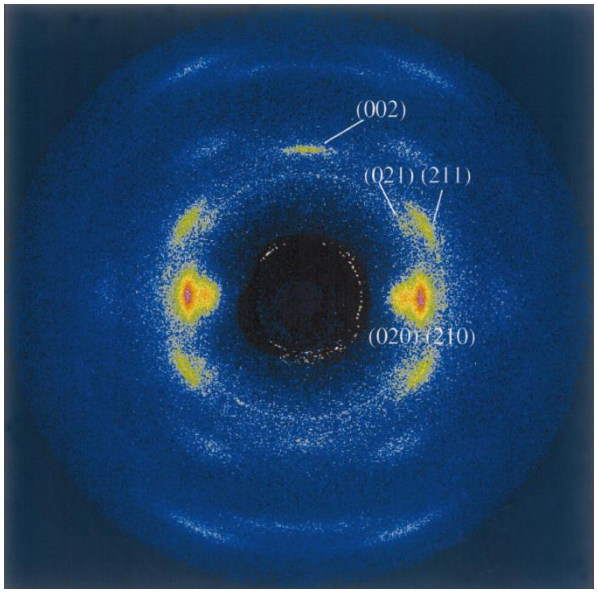

Figure 6. X-ray diffraction pattern of a single fibre of *N. clavipes*. X-ray beam is oriented normal to the fiber axis. The Miller indices of the different reflections are indicated directly on the image and show Bragg reflections, an oriented amorphous halo and a general diffuse background. The data are consistent with the presence of two fractions of different crystallinity and an amorphous phase. From Riekel et al. *Int J Biol Macromol* **24** (1999) 179, permission required.

As often in nature, the actual structure of MA silk is more complex than the above two-phase description. Several studies indeed provided various evidences that a third phase, called *interphase*, composed of intermediary ordered and/or oriented structural elements (possibly amorphous β-sheets), links the crystals and the disordered chains.[60,61,95-100] This intermediate phase would be important to understand silk mechanical properties. The proportion of the interphase seems smaller for *B. mori* silk.[65] It has been suggested that the amorphous phase would be in a constrained state according to a Gaussian distribution of pre-strained chains that would critically control silk mechanical response.[101,102]

Another model based on linear viscosity theory confirms the role of the disordered matrix on silk's extensibility, but shows that the stiff crystals are also elastically deformed upon stretching.[103] Then, the dense H-bonding assembly of β-sheets seem to overcome the intrinsic weak strength of H-bonds and

enhance the rupture stress of silk.[104-106] Indeed, it has been found that the rupture of β-sheets should involve the breaking of a maximum of 3-4 H-bonds simultaneously. This finding leads to an "intrinsic size limitation" suggesting that strands of small lengths such as those found in spider silk respond with the highest cooperativity.[104] Subsequent molecular dynamics simulations have shown that β-sheet nanocrystals achieve a higher stiffness, strength and toughness when restricted to a few nanometers as compared with larger nanocrystals.[105] Moreover, hydrogen bonds of small crystals subject to deformation can reform according to a "stick-slip" mechanism before complete rupture, thus exhibiting a self-healing ability.[105] A representation of the hierarchical structure of MA silk and a description of the structural origin of the mechanical properties are presented in Figure 7 and 8, respectively.

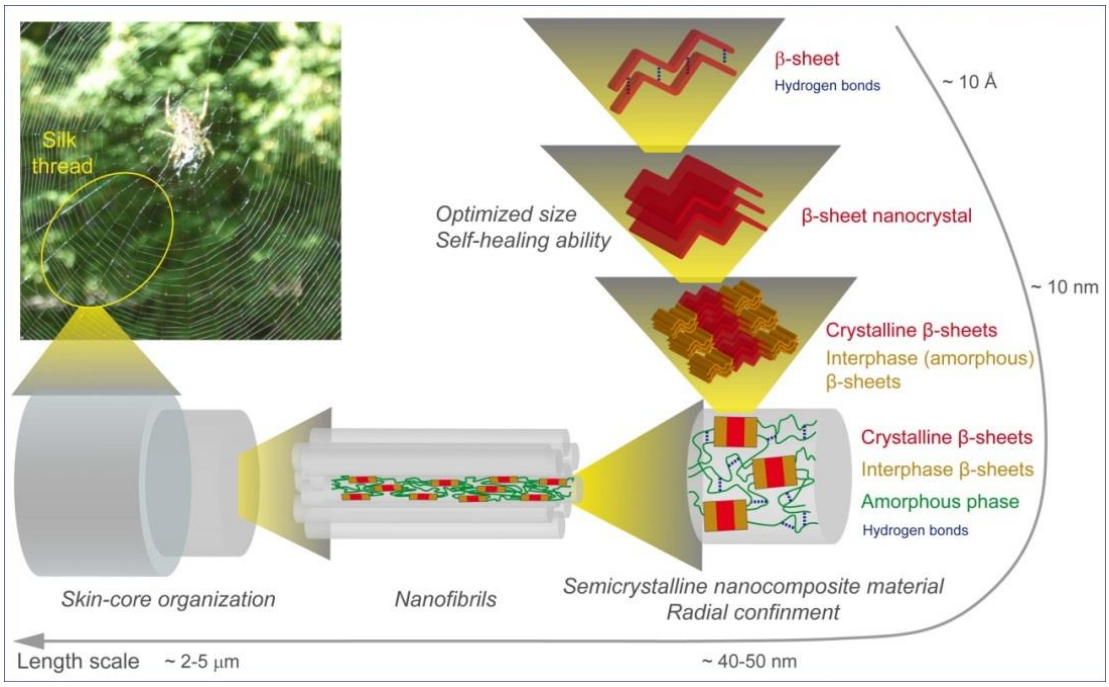

Figure 7. Schematic representation of the hierarchical structure of dragline silk. Various structural elements and properties that contribute to silk resistance are underlined. Adapted from Keten et al. *Nat Mater* **9** (2010) 359.

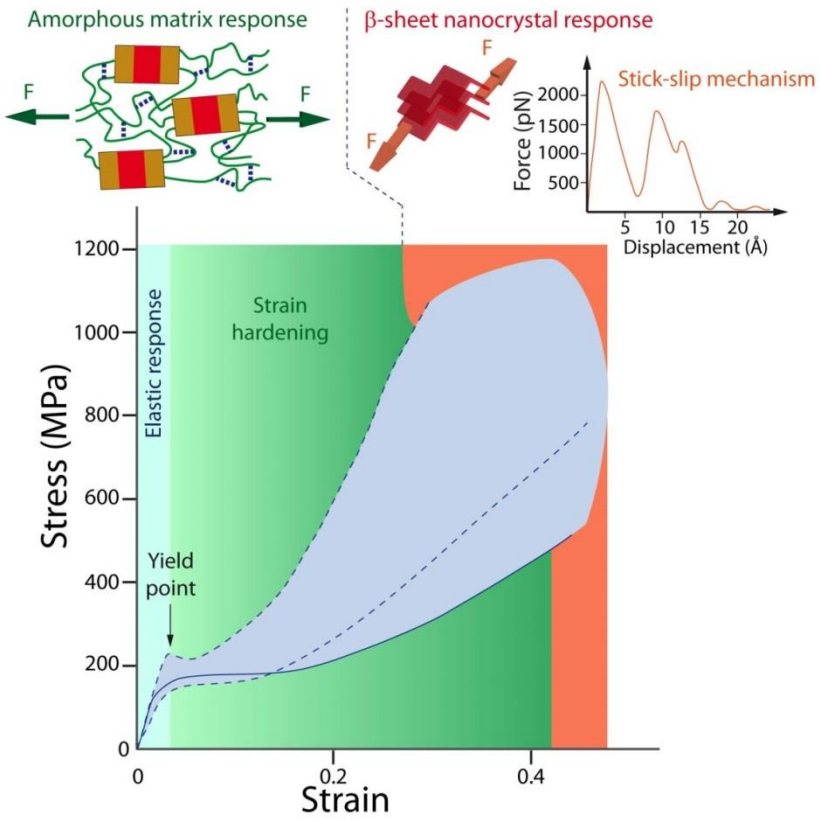

Figure 8. Relation between structure and tensile properties of MA silk. Various strain-stress traces obtained form naturally spun fibers frrom *Argiope trifasciata* are represented. They delimit a blue area illustrating the biological variation of the natural fiber (traces redrawn from Elices et al. *J Mech Behav Biomed Mater* **4** (2011) 658). The mechanical behavior of silk is non linear in a multi-regime fashion. The response of the fiber at low strain is dominated by the amorphous phase whereas at high strain the response of the fiber is mainly due to β-sheets. The short elastic regime corresponds to a homogeneous and reversible deformation of the amorphous matrix. This is followed by a more or less apparent plateau where the amorphous domains/chains untangle with the breaking of intermolecular hydrogen bonds. As the polypeptide chains are extended, β-sheet crystals are involved and submitted to stresses resulting in an increase of the slope of the curve (strain hardening). Just prior breaking, a slip-stick behavior of the β-sheets deduced from atomistic simulations may extend even more silk resistance to elongation (adapted from Nova et al. *Nanoletter* **10** (2010) 2626 and Keten et al. *Nat Mater* **9** (2010) 359).

The structural organization at higher length scales may also contribute to mechanical properties. It has been suggested that the MA filament exhibits a skin-core structure (Figure 7).[73,107-109] MaSpI has been found uniformly distributed within the fiber whereas MaSpII would be concentrated in clusters within the core of the fiber and less present in the periphery.[73] Several data also points towards a microstructure composed of nanofibrils (Figure 7).[82,92,109-114] This observation is supported by model simulations based

on a coarse-grain model that showed enhanced strength, extensibility, and toughness when the structural organization is spatially confined below radial lengths of 50 ± 30 nm, which seems to synergistically optimize the action of protein domains.[115] The nanofibrils further seem to be twisted, which would provide a mechanism to inhibit crack propagation during tensile elongation.[116] The hierarchical model established from these "bundle" of intertwined fibrils allowed to predict the breaking stress of spider dragline and silkworm cocoon silks[116] using the order and density of the β-nanocrystallites as relevant parameters.

MA silk appears overall constituted by relatively simple components as spidroins are mainly constituted by simple amino acids (glycine alanine. serine).[117] However, the amino acid arrangement with modular motifs it is still more complex than synthetic copolymers. This apparent simplicity of the raw materials results in high-performance fibers which in fact relies on a highly complex and optimized organization structured at all length scales. Hierarchy appears as a fundamental requirement to achieve high toughness materials.[118] Silk structures result from the spider behavior-driven processing of protein solutions under specific aqueous conditions in a complex system of abdominal glands of various morphologies. Mimicking silk will thus require the capacity to reproduce equivalent or similar architectures. To date however, such a control of the final organization at all length scales is far from being routinely attainable in materials science.[119] But the diversity of structural arrangements and characteristics may prompt one to suggest that appropriate design of protein sequences and suitable spinning processes can lead to the production of synthetic fibers with striking, diversified and tailored properties.

2.5 Other properties of silk

Besides its remarkable tensile response to drawing, MA silk possesses other mechanical properties that may be exploited. It displays for instance peculiar torsional characteristics, including a shape memory behavior[120] and super-elasticity, i.e. silk's capacity to reversibly withstand great torsion strains of over 10^2-10^3 rotations per length unit before failure.[121] These mechanical features have been very slightly investigated but the understanding of the structure-property relationship would certainly be very fruitful in materials science.

Some silk fibers such as MA silk are also particularly sensitive to water. When wet or in a high relative humidity atmosphere, they are subjected to a longitudinal shrinking of up to 60% of the length of the fiber concomitantly with radial swelling (about two-fold increase of the diameter).[122-125] This phenomenon, so-called *supercontraction*, is reminiscent of what occurs for oriented synthetic polymers above their glass transition temperature. It is due to a plasticizing effect of water molecules that insert within the hydrogen bonded polypeptide networks and reduce intermolecular interactions, thus providing polypeptide chains with mobility which in turn allows chain entropic recoil. Being very flexible amino acids, glycines seem to be particularly efficient to disorder protein backbones in the presence of water (i.e. in the absence of interchain H-bonds).

It turns out that the situation where the fiber is maximally supercontracted can be obtained independently from loading history of the fiber, even when stretched to large deformations. Therefore, the "maximum supercontracted state" can be viewed as a ground (reference) state for silk where the chains are minimally aligned and constrained.[126-128] The amplitude of supercontraction depends on the type of silk and spider species. For example, the maximum supercontraction of *B. mori* fiber is 3%,[129] that of the moth *Antheraea Pernyi* is 5%[130] while minor ampullate silk do not shrink at all.[122,131] The shrinking mechanism has been related to the Pro residue content,[132,133] although this amino acid has been found not to be essential for the occurrence of supercontraction.[134]

Finally, MA silk also exhibits diverse characteristics including specific optical qualities,[135,136] vibration propagation properties[137] as well as thermal[138,139] and electric[140] conductivity. Overall, natural silk appears to be endowed with striking and inspiring physical properties that rely on a complex hierarchically organized nano-composite structure. Although recent works suggest that the high tensile strength of spider silk may simply rely on a general rule specifying that low diameter fibers are stronger than larger ones,[141] the diverse structures formed at different length scales and their arrangement determine the ensemble of attributes that make silk a versatile and astonishing material.

3. Spinning artificial fibers

3.1 The natural spinning process

Being part of nature, the spinning process of silk is a model of impressively optimized and low-footprint mechanism that may inspire researchers. Both spiders and silkworms convert in the timescale of less than a second a highly concentrated and viscous protein aqueous solution into an insoluble solid fiber. Whereas silkworms excrete the cocoon silk by salivary glands located in the head region, spiders produce silk in abdominal glands. Orb-weaving spiders spin filaments from glands with various morphologies (Figure 1). As an archetypal example, the MA excretory system can basically be divided into three regions called the tail, the sac and the duct (Figure 9). The tail is a long tubular region where the spidroins are synthesized. The sac is an ampulla where the spinning dope is stored before use. The duct is composed of three limbs with a tapered shape folded on themselves into a flat S shape (unfolded in Figure 9), in which the native liquid silk is progressively transformed into a fiber. The duct ends with the spigot where the filament is pulled from the exterior.

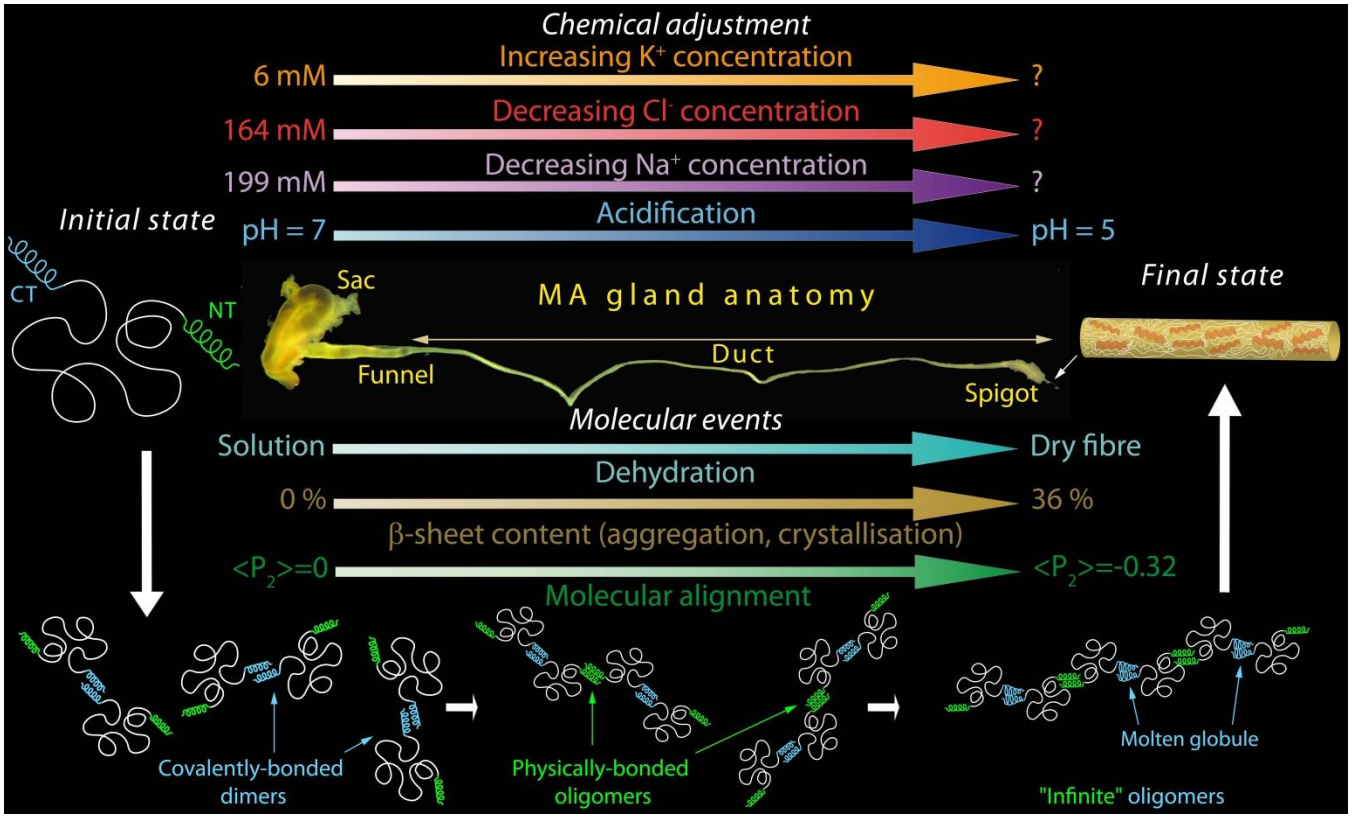

Figure 9. Schematic representation of the natural spinning of spider MA silk and chemical adjustment of the aqueous dope. An image of the MA gland of *N. clavipes* is shown in the centre of the figure with its natural yellow colour. The duct is in a non-native (developed) configuration. Chemical adjustments and molecular phenomena occurring along the gland are represented by coloured horizontal arrows in the upper and lower part of the figure, respectively. Initial and final values of relevant parameters are given (when known) at the left and right sides of these arrows, respectively (ion concentrations and pH values are taken from Rising & Johansson *Nat Chem Biol* **11** (2015) 309, β-sheet content and $\langle P_2 \rangle$ values are taken from Rousseau et al. *Biomacromolecules* **5** (2004) 2247). The initial (solution) and final (fibre) molecular states of the spidroins are presented on the left and right sections of the figure, respectively. The CT and NT α-helix sequence folds are represented by light blue and green single helix symbols, respectively. The assembly mechanism as influenced by pH is described by the sequence of events related by white arrows (adapted from Rising & Johansson *Nat Chem Biol* **11** (2015) 309).

3.1.1 Molecular transformations during spinning

The dope solution contained in the sac of the gland is highly concentrated (30-50 % w/w),[142,143] and stable in vivo. At this stage, MA spidroins are mainly unfolded[142,144,145] with the presence of 3_1 helices and random coils.[146] It has been proposed that spidroin tertiary arrangement lead to the formation of micelle-like globules in which the more hydrophobic polyalanine blocks form a core and the hydrophilic glycine-rich blocks are exposed to water.[147] This colloidal structure has recently been supported by mesocopic modeling.[148] These globules would aggregate, elongate and align as the dope flows within the duct of the gland. The molecular weight and the relative size of the hydrophobic block as compared to the hydrophilic one affect the size and structure of the globules as well as the assembly process.

Weaving, falling and walking are various circumstances where silk is produced. Animal movements result in the application of physical constraints on the spinning dope that are at the origin of silk formation.[122,149] Since the spinning dope is a viscoelastic fluid before becoming a solid and water-insoluble thread, the mechanical drawing is more appropriately called an extensional flow. In addition to this driving force, shear stresses are applied on the dope during the flow in more proximal regions of the gland against the luminal wall of the duct. This shear stress seems to be at the origin of the formation of precursory β-sheets although without molecular alignment.[150] More extensive changes occur near the *draw down taper*, a region located near the spigot where the lumen is peeled off from the duct wall. This region is the seat where the dope is submitted to a large extensional flow. The applied stress induces polypeptide chain orientation along the elongation axis,[6,150-152] promotes intermolecular interactions and β-sheet formation[150,153] while water is phase-separated,[151-154] leading ultimately to the final complex hierarchical organization of the spidroins within the fiber as we know it. The process is influenced by the environment[155-157] while silk chemical structure[158] and mechanical response[155,159] are affected by the animal's diet and behavior (reeling speed).[92,157]

The duct exhibits a specific internal geometry which is thought to contribute to the efficiency of the spinning process. The internal secretory pathway can indeed be represented by a two-stage hyperbolic dye, resulting in a slow reduction in diameter that is thought to induce a low extensional flow rate, which in turn may inhibit premature crystallization of the proteins.[160] The internal draw-down taper exhibits a different diameter profile as it can be fitted with a double exponential, supporting the idea that the extensional flow is much higher in the distal part of the duct than in more proximal regions.[160] A similar spinneret profile has been observed for the spinning apparatus of *B. mori*[152] and for the wild *Samia cynthia ricini*[161] silkworms. Distally from the draw down taper of spiders arises a muscular organ designated as the *valve*, which is thought to act as a clamp to hold the thread more or less tight and control the pressure applied on it, and allows to continue spinning in case of an internal breaking of the filament.[162] The epithelium of the distal section of the duct is also equipped with specialized water-pumping cells that help in removing exceeding water.[6] It has been proposed that the spidroins in the dope are initially assembled within micelles that subsequently aggregate into larger clusters and ultimately form a gel state.[163] Such geometrical and biological details may be useful to the design of synthetic silk-forming devices.

3.1.2 Rheological properties

The spinning dope is metastable and can easily be converted into a β-sheet-rich solution, by stretching for example. Due to the high molecular weight of the proteins and due to the high protein concentration the spinning dope is highly viscous (~ 3.5×10^6 that of water [151]). Therefore, to be spun into a solid fiber just from the force applied by the spider's body weight or by the animal's traction, nature has provided the spinning dope with particular viscoelastic properties. Several studies have found that the spinning dope of the MA gland is in a liquid crystalline state.[160,164,165] Similar findings have been obtained for the fibroin solution.[152,166,167] Differences in the texture of the dope (nematic, cholesteric, discotic

mesophases) have also been noted.[6,152,164] They may be related to the positions along the secretory pathway and/or to the hydration state of the solution.[164,166,168]

Liquid crystallinity is thought to promote the spinnability of the dope despite its high protein concentration. Liquid crystallinity indeed provides the dope with non-Newtonian rheological characteristics, including a high shear-thinning capacity (a decrease of the viscosity with increasing shear rate),[143,151] thus making drawing more energy-efficient and promoting orientational ordering.[6,151,164] The native silk dope of *B. mori* consistently exhibits shear thinning in oscillatory rheological experiments.[169-171] The spinning dope also displays shear thickening[143] and strain-hardening during extensional rheology experiments performed with a capillary break-up micro-rheometer, so that the apparent extensional viscosity diverges at large strains and, ultimately, the viscoelastic dope solution dries to form a solid fiber with a finite and uniform diameter.[151] This strain hardening, due to the combined action of molecular alignment and water evaporation, is thought to contribute to the stabilization of the spinline and the inhibition of capillary break-up of the fiber during extensional flow.[151] Interestingly, *B. mori* and spider MA spinning dope exhibit similar shear-stress rheological behavior and seem to behave like polymer melts.[172]

Thus, different factors such as the physical state and rheological behavior of silk spinning dopes are finely optimized to make the natural spinning process highly efficient. In this respect, it has further been found recently from rheological measurements that the natural spinning requires much less energy than usual synthetic fiber-producing processes as illustrated in Figure 10.[173] According to this study, the industry sector might be able to reduce the energy costs related to the production of polymer fibers by over 90% if scientists demonstrate their ability to reproduce a similarly optimized process.[173]

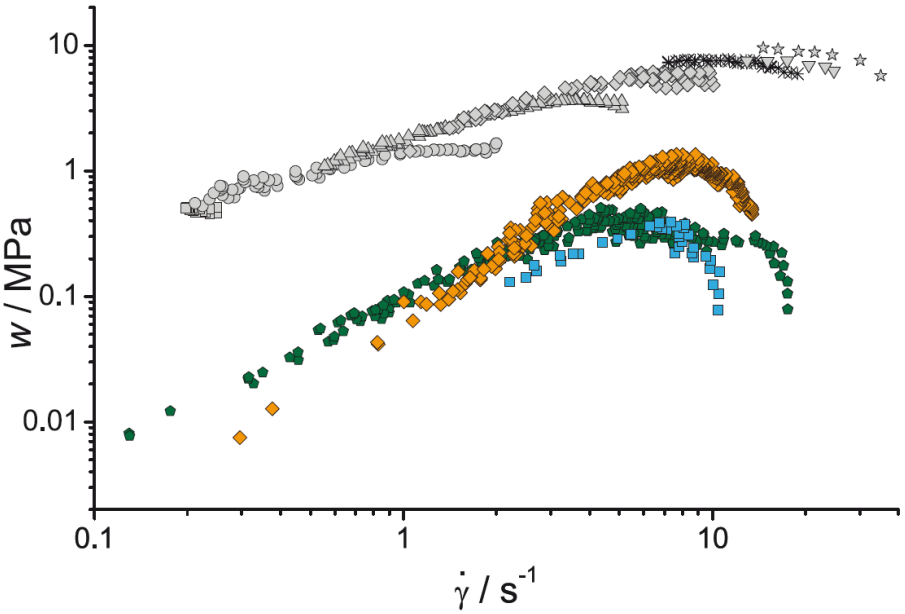

Figure 10. Rheological data showing the log–log plot of the specific work of gel formation (*w*) as a function of shear rates ($\dot{\gamma}$) for silk at different temperatures (10 °C (blue squares), 27 °C (green pentagons) and 40 °C (orange circles)) as compared to HDPE at 125 °C (grey symbols). The silk

requires over an order of magnitude less energy to initiate fibrillation at a given shear rate. (From Holland et al. *Adv Mater* **24** (2012) 105, permission required).

3.1.3 Chemical adjustments during spinning

In addition to physical treatments, the lumen is submitted to specific evolving chemical conditions along the duct, including pH, salt concentration, type of salt (Figure 9). The aqueous environment seems thus to be finely tuned to optimize the spinning process. From the ampulla to distal area of the third limb of the duct, the pH has been found to decrease from 7.2 to 6.3[154,174,175] (Figure 9) and may even drop to more acidic values.[176,177] It seems that the pH gradient is produced and controlled along the gland though carbonic anhydrase enzymatic activity, by catalyzing the reaction $H_2O + CO_2 \leftrightarrow H^+ + HCO_3^-$.[53,178] Moreover, metallic Na^+ and Cl^- ion concentrations drop ten and fourfold in dry weight along the secretory pathway, whereas K^+ and phosphate ions increase fourfold and fivefold, respectively.[174,179] Rheological behavior,[143,171] spidroin conformation[175,180] and tertiary structure[177,181] seem to be specifically reactive to pH changes. Higher chloride concentration stabilizes the native conformation while salts and pH modulate the aggregation mechanism of spidroins.[52,177,182]

The variations of the aqueous environment seem to have profound and peculiar repercussions on the stability and behavior of the NT and CT regions that in turn play crucial roles in the spinning process and stability of the dope.[53] NR domains are mainly α-helical in their native environment. The NT domain is known to associate to form stable homodimers via physical bonds at pH below 6.4, the monomeric form predominating above pH 7.[176,183-185] This phenomenon has been rationalized at the residue level using molecular dynamics.[186] Such a dimerization propensity at low pH may critically affect the protein assembly by acting as an intermolecular crosslinker.[177,187] As a matter of fact, aggregation properties of MA spidroins seem to be regulated by pH-response of the NT region, as recombinant spidroins bearing the NT moiety avoid aggregation above pH 7 (in storage-like conditions) and rapidly self-assemble at pH 6 (in conditions associated with distally-distanced area of the spinning duct).[48]

The CT domain of MA silk forms disulfide-linked dimers. Then, the covalently-bonded CT domains in conjunction with stable physically-bonded NT domains may theoretically assembles into successions of spidroins to form infinite oligomers.[177] Depending on pH, the CT domain seems to adopt a particular molten globule conformation that may promote further spidroin assembly by acting as a nucleus for β-aggregation.[49,178,188] The CT domain seems to be essential to the spinning process as spidroins that lack this region form amorphous aggregated rather than well-ordered filaments.[51] Its response to pH also triggers the conversion from a soluble, storage-adapted form to an aggregation-prone form.[49]

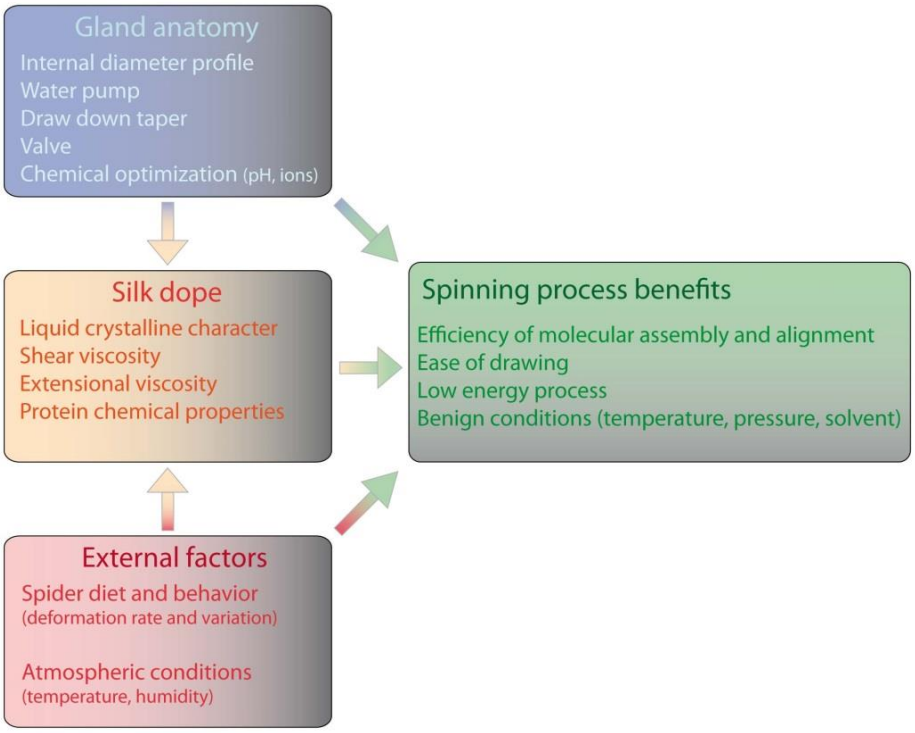

Figure 11. Relation between the gland anatomy, spinning dope and external determinants that contribute to natural spinning process, and their potential benefits for the production of synthetic spidroin-based materials.

Having been optimized for hundreds millions years of evolution, natural silk spinning proves overall to be a complex, finely-tuned series of multi-domain molecular events with adjusted physical and chemical variables and complex machinery (summarized in Figure 11) that together lead to the formation of an astonishing hierarchical nanomaterial composite. It also appears as a low energy-demanding and ecologically optimized process that reduces the metabolic cost of silk production.[6,189] Since, in addition, natural silk is manufactured in mild temperature and pressure conditions, in water, i.e. without the use of harmful organic solvents such as those used for the production of Kevlar, mimicking spiders' spinning process would potentially lead to the production of human-made materials with a lower ecological footprint. Therefore, the full practical and environmental benefits of the attractive qualities of spider silk will depend on our capacity to successfully mimic the natural spinning in an industrial context and control the entire process and final architecture.

3.2 Synthetic silk spinning processes

Many efforts have been devoted to the fabrication of artificial silk, many aiming at reproducing the tensile properties of the MA silk. One of the challenge regards the availability of the spidroins. Because spiders are cannibal and territorial and because they synthesize small amounts of silk, the production of spidroins by biotechnology techniques is regarded as the most efficient and economically viable means for the supply of spidroins.

Expressing spidroins in appropriate hosts (bacteria, plants, insects or mammals) may also appear attractive from an environemental perspective since recombinant proteins represent renewable feedstocks. However, depending on the host chosen, these methods may have various environemental repercussions (water and energy use, agricultural land or rearing requirements) and require physical or chemical treatments of the wastes and byproducts to avoid potential health hazards (pathogenic, toxic or allergenic contamination). These shortcomings have to be taken into account for environmental and social considerations. Since these issues have been discussed elsewhere,[190] and for brievity reasons, they will not be further discussed here.

It is noteworthy that one of the limitations in mimicking silk concerns the difficulty in expressing spidroins with the same molecular weight than natural ones. As a matter of fact, the high molecular weight of natural proteins is viewed as an important determinant to match the tensile property of the natural fibers.[148,191,192] Native-sized recombinant spidroins have recently been successfully expressed.[193,194] Interestingly, synthetic fibers made with these proteins could achieved mechanical properties close to those of the native fiber.[193]

Although recombinant spidroins with the native sequence is important, the use of related (fusion or hybrid) proteins is often chosen as an alternative avenue to design innovative biomaterials. Then, although the protein primary structure is essential for silk structure, the way the protein solution is processed is crucial as well.[33,126,195] This conclusion is illustrated by the fact that the solubilization and reprocessing of native filaments do not lead to fibers with comparable tensile properties than natural ones.[196,197] Also, researchers experience difficulties in processing spidroins in aqueous solution and often resort to organic solvents. These failure represents to date some of the main limitations of the endeavors to mimic natural silk and implement environmentally friendly spinning processes. In the following, the different methods used for synthetic spinning and their advantages are presented.

3.2.1 Wet spinning

Wet spinning is the conventional procedure to generate artificial fibers and first consists in the solubilization of spidroins, either recombinant proteins or native fibers, in some solvent. Due to the high crystallinity and strong cohesive bonding nature of silk proteins, the solubilization of silk fibers requires highly denaturing conditions such as concentrated chaotropic aqueous solutions or specific, often fluorinated, organic solvents.[198,199] This requirement may also hold true to solubilize recombinant proteins that often are recovered in the form of strongly bonded and compact precipate powder. In the case of denaturing aqueous solutions, the salt has to be removed using dialysis or elution through salt-exchange desalting columns.

Solutions are subsequently injected into a coagulation bath and drawn, which results in the precipitation and alignment of the polypeptide chains with the conversion of protein sequences into β-sheets, to finally form a solid filament. The fiber so obtained can further be post-drawn,[126,196,200-203] a treatment that is one of the favored and most efficient strategies to increase the level of molecular orientation and improve materials' mechanical response. The formation of any spidroin-based material follows essentially the same procedures than those used for the production of fibers *in vitro*. Proteins are

generally first solubilized in a highly concentrated salt aqueous solution or in organic solvent, dialyzed if necessary, and subjected to a coagulation treatment to provide cohesiveness to the sample.

Most of the attempts to produce regenerated fibers have been carried out with *B. mori* fibroin, in particular using concentrated inorganic or organic salt solutions,[198,199] organic or acidic solvents (such as hexafluoroisopropanol (HFIP),[201,204-209] trifluoroacetic acid (TFA),[210] formic acid[210,211] and hexafluoroacetone (HFA) hydrate[212]), or both. However, the use of salts, although often unavoidable to solubilize silk fibers, may degrade the proteins[213,214] and requires long dialysis times, which represents an economic limitation, while organic solvents should be avoided for wealth, environmental and/or economic reasons. In addition, it may further be hypothesized that using the natural aqueous environment might be the best way to reproduce the natural spinning process and obtain materials comparable to native silk.

Spinning fibroins and spidroins in water has been carried out using purified recombinant proteins or solubilized fibers. The first problem one encounters with silk protein aqueous solutions regards the fact that the solution is unstable and tends to aggregate spontaneously. It is thus generally difficult to maintain the spidroins in solution, especially at high concentration.[215] To mimic natural conditions, high concentrations may be required although it has been found that 15% is an optimal concentration for synthetic spinning. It seems in any case difficult to exceed 20-25 % w/w and, for the moment, difficult to reach the native concentration found in the gland ampulla and to keep the solution stable. High concentrations may however be required to reproduce the liquid-crystalline character of the natural dope.

Consistently with the metastability of silk protein aqueous solutions, rheological studies have shown that regenerated fibroin solutions is sensitive to shear[216-219] and pH[171] such that the protein undergoes aggregation or gelation with the formation of β-sheets. Solutions formed *in vitro* do not exhibit the same rheological characteristics than the natural spinning dope contained in the gland, which seems to arise from a loss of protein integrity during the dissolution process, especially a reduction of the protein molecular weight and change in conformation.[220,221] The impact of the protein conformation on rheological properties has also been emphasized comparing the native silk dope of *B. mori* with the content of the MA gland[169] or other silkworms.[170]

Natural *B. mori* cocoon and spider MA fibers[196,197,208] as well as recombinant proteins[202] have been dissolved in aqueous solution and processed to form fibers.[200,222] The coagulation bath is most often constituted of neat methanol[201-204,207,210] but methanol aqueous solutions,[202,203] acetone,[196,208] 2-propanol[208,209] and an aqueous solution of ammonium sulphate[200,222] has also been used. Various parameters such as coagulant composition, coagulation rate and temperature have been investigated and have been found to influence the morphology of the final fibers.[211,222] The coagulation rate has been found crucial to achieve good mechanical efficiency.[222] This conclusion is consistent with the fact that the solvent conditions strongly influence the kinetics of protein aggregation assembly and can lead to protein aggregates that exhibit very different molecular arrangements, shapes and textures.[223,224]

3.2.2 Dry spinning and other methods

Procedures mimicking more closely the natural process have been reported. These methods, called *dry spinning*, do not use a coagulation bath and consist in allowing the spidroin aqueous solution for drying and pulling it to form a solid filament.[197,198,225-227] Porous silk fibroin matrices have also been successfully formed without the use of organic solvent by adding gelatin to the aqueous silk fibroin solution and by water annealing [228]. This method is thought to optimize hydrophilic interactions in the silk fibroin-gelatin-water system, thus restraining the formation of β-sheet structures, which in turn results in a more homogenous organization.[228] Spidroins often exhibit a propensity to self-assemble in solution with may be exploited to form fibers.[50,51,229,230] As a matter of fact, reasonable tensile strength has been obtained in these conditions, i.e. without applying any spinning procedure, as compared to fibers spun from dissolved natural silk.[50] It is finally noteworthy that a totally different strategy has been developed to mimic nature. It consist in using transgenic *B. mori* silkworms as a biological spinning machinery to directly produce fibers made of spidroins.[231] From an industrial point of view, this method would therefore require to obtain recombinant spidroins with biotechnologies and to breed transgenic worms as processing devices.

3.2.3 Microfluidics

Microfluidic approaches appear as potentially efficient methods to implement a fiber formation process that captures certain (all?) aspects of the natural spinning process, and that allows the control of several parameters of the aqueous environment such as pH decrease or ion exchange.[53,232-236] Channel dimensions and flow rate allow controlling fiber diameter and spinning speed.[232] Various device configurations have been employed to be as close as possible to the duct geometry and spinning conditions. Double[234,236] or triple[232,235] inlets, either in Y junction or concentric configurations, allowed the flow of the aqueous spidroin solution to be mixed with mobile aqueous phases that reproduce pH or ionic changes occurring in the MA gland[235,236] or match the viscosity of the dope.[232] A coagulation bath can again be used to induce protein assembly[232] but the extension flow taking place in nature can be mimicked with appropriate geometry[235] Another device consists of a laminar flow formed of a mobile oil phase and a spidroin solution flowing in parallel, which induces the self-assembly of the proteins and the formation of a fiber at the interface between the two phases.[233] Microfluidics then represents one the best methods to implement a spinning process as close as possible to the natural one.[53,232] It constitutes also a means to better understand the relationship between spidroin sequence, spinning process and the final fiber structure.[232] Finally, it may lead to the design of customized biomaterials, for instance to long fibers.

3.2.4 Successes and difficulties in mimicking or equaling nature

Plentifully benefiting from the potential advantages of spidroin-based materials will depend on our capacity to achieve green processing procedures that lead to materials with mechanical properties similar to the native fibers. This obviously appears as a complex task and many works are still needed especially regarding the understanding and the control of the behavior and self-assembly abilities of spidroins. Scientists are far from controlling all aspects of the final structure and understanding the relationship between the processes applied and the detailed organization of the product formed. Recent remarkable advances, however, prove that scientists are making progresses,[24,237] especially with *B. mori*

silk. For example, some of the "green" advantages of silk (processability in water and at room temperature) have been exploited to produce silver nanoparticles using light and fibroin as template. Using an environment-friendly and energy-saving procedure, the resulting silk fibroin-silver nanoparticle composite film has an effective antibacterial activity against *Staphylcoccus aureus* and inhibits biofilm formation.[238]

Table 2. Mechanical properties of *B.mori* and spider silk-inspired fibers compared to natural fibers.

Materials/sample preparation		**Young modulus (GPa)**	**Strength (GPa)**	**Strain (%)**	**Toughness (MJ/m^3)**	**Ref.**
Natural B. mori fiber (degummed)		*7*	*0.50-0.60*	*15-18*	*70-80*	
Regenerated *B. mori* fibroin	Wet spinning (coagulation bath: ammonium sulfate aqueous sol.)	n.d.	0.26-0.45	28-79	100-149*	[222]
Regenerated *B. mori* fibroin	Wet spinning (MeOH coagulation bath, post-drawn in water)	12.2	0.32	6	n.d.	[195]
Regenerated *B. mori* fibroin	Dry spinning (post-drawn in EtOH-water)	3.9	0.15	31	33	[239]
Regenerated *B. mori* fibroin	Dry spinning (post-drawn in EtOH-water)	6.8	0.20	55	79*	[227]
Regenerated *B. mori* fibroin with multiwalled carbon nanotubes	Wet spinning (ammonium sulfate coagulation hbath)	n.d.	0.20-0.42	52-77	107-186	[240]
Regenerated *B. mori* fibroin with TiO_2	Wet spinning (alcohol coagulation bath, post-drawn)	2.9-7.0	162-219	32-88	38-93	[241]
Natural (forcibly spun) N. clavipes MA silk		*13.8*	*1.22*	*19*	*111*	[31]
Regenerated *N. clavipes* natural silk	Wet spinning (dissolved in HFIP, acetone coagulation bath, soaked in water, post drawn in air)	8	0.32	n.d.	n.d.	[196]
Recombinant *N. clavipes* spidroins (MaSp1 and MaSp2)	Wet spinning (isopropanol coagulation bath, post-drawn)	5.6-7.7	0.28-0.35	27-42	55-72	[209]
Recombinant *N. clavipes* Spidroin (native-sized MaSp1)	Hand drawn, solublised in HFIP	21	0.51	15	n.d.	[193]

Chimeric silkworm/MA silk protein	Transgenic silkworm spinning	5.0-5.5	0.28-0.34	31-32	69-77	[231]

* Assuming a density of *B. mori* silk of 1.35 g/cm^3.

If the development of a green spinning process is a challenging objective, achieving materials as performant as natural silk is complex as well. Despite many efforts and promising results, biomimetic strategies to reproduce native-like fibers with an equivalent mechanical resistance reveal relatively unsuccessful to date (Table 2).[197,198,202,203,209,242-244] Some works proved that scientists can approach the characteristics of native fibers. For instance, recombinant proteins MaSpI and MaSpII from *N. clavipes* allowed producing fibers with a globally similar structure and close tensile performances than natural silk except at large deformations.[209] By a close control of the rate of protein coagulation, similar tensile properties have been achieved for regenerated *B. mori* silk dissolved in aqueous solution.[222] But despite these remarkable advances, scientists have not achieved a total control of the structure and mechanics 8of the fibers. At the present time, reinforced hybrid silk materials appear as the best strategy to develop fiber with comparable tensile properties than spider silk (Table 2). Synthetic processes also seem to result in final materials that exhibit variabilities in their tensile property from batch to batch, which may represent a major shortcoming for applications.[245] It proves difficult to reproduce all the chemical and physical subtleties of the natural spinning mechanism while several problems must be solved before this system becomes commercially viable. A better understanding and control of the natural spinning process and effect of various parameters, including the aqueous environment will probably lead to improve mechanical resistance of the fibers formed.

4. Biological properties

4.1 BIOCOMPATIBILITY

Silk is not only attractive for its mechanical properties but also for some biological attributes. *B. mori* silk has been used as biomaterials for wound healing for centuries.[26,246] Today, it is widely employed in the textile industry and medical suture field whose current demands are satisfied by the sericulture industry in developing or emerging countries.[237,247] Novel applications in the biomedical sector rely on the biocompatibility of silk-based materials. Biocompatibility encompasses various characteristics, including the absence of toxicity, inflammatory and immunogenic responses (*biosafety*) on one hand, and the capacity of cell adhesion and proliferation (*biofunctionality*) on the other hand.[248]

4.1.1 Biosafety

Any exogenous biomaterial implanted in living tissue will generally lead to physiological and biological reactions as well as progressive molecular events at the interface between the implant and the surrounding medium.[248] Native fibers, synthetic materials as well as their *in situ* degradation products may potentially induce adverse biological responses.[246,248] Various factors can affect individual reactions

such as implantation site, protein sequence, size, geometry, surface-to-volume ratio and surface features of the biomaterial. Hypersensitivity may be enhanced by repeated exposure to an exogenous biomedical component.[246]

In the case of silkworm silk, various studies have shown that silk fibroin can potentially be allergen or inflammatory although sericin is the most probable constituent at the origin of the reaction.[246] However, an adequate removal of any trace of sericin may make *B. mori* silk an appropriate material for biomedical applications. Contaminants have also been found to be at the origin of body defense reactions.[246] Potential risk factor for amyloidogenesis has been raised but no amyloidogenic problem has been reported to date for silk-based biomedical systems[246,249] except for *B. mori* fibroin that has been shown to accelerate amyloid accumulation *in vivo*.[249]

Being devoid of the sericin glue, spider silk does not seem to suffer from similar problem than silkworm silk. Due to the reduced availability of the raw material however, only a few studies have been devoted to the biosafety of spider silk. The literature actually shows diverse reactions and severity of the body response when natural or synthetic spider silk is implanted in living tissues. This disparity could probably be rationalized if one considers the diversity of materials used.[248] However, it is hypothesized that the low-complexity and expected low immunogenicity of the main nonrepetitive region of the spidroin primary structure may induce weak antibody response.[248] More works regarding the biosafety of natural and synthetic spidroins are required to ascertain their inoffensiveness.

4.1.2 Biofunctionality

Overall, spidroins prove to be promising building blocks to induce the adhesion and growth of cells for tissue engineering and to replace tissues such as bones, cartilages ligaments, tendons or nerves.[250] Interestingly, MA spidroins naturally contain RGD cell-binding domains,[251] like fibroins of some non-mulberry silkworms.[252] Pristine spider silk fiber has been used as templates to accommodate seeding and proliferation of fibroblasts[253] and to form nerve conduits thanks to the growing of Schwann cells.[254,255] It was also suitable for the formation of oriented nucleation of hydroxylapatite crystals for bone implant applications.[256] The orientation of the hydroxylapatite crystals has been suggested to be driven by β-sheet nanodomains,[256] suggesting that the morphology of the fiber surface may influence cell adhesion.

Synthetic scaffolds made of several recombinant silk protein constructs have been successful in supporting the development of various cells, including fibroblasts,[257,258] neural stem cells (NSCs)[259] and chondrocytes for cartilage regeneration.[260] Surface parameters of scaffolding such as charge, wettability and topography are important determinants for the efficiency of cell adhesion and growth.[261] As an example, it has been shown that the patterned structure of silk scaffolds is important for cell adhesion and orientation.[262] The amino acid composition of spidroins can also influence cell adhesion and proliferation.[248] As a matter of fact, charged side-chains and alternating hydrophilic and hydrophobic motifs such as those found in natural dragline silk are thought to promote cell adhesion.[248]

Recombinant proteins are thought to be advantageous over natural protein or synthetic polymer scaffolds due to the possibility of designing diverse chimeric sequences with desired and controlled functionality thanks to genetic manipulations.[263] For example, bioengineered protein variants constituted

by the combination of the consensus sequence of MA spidroin from *Nephila clavipes* and RGD cell-binding domains were designed to improve the culture of human bone marrow derived mesenchymal stem cells upon addition of osteogenic stimulants.[264] A similar strategy has been used for the attachment and proliferation of fibroblasts using protein constructs based on the sequence of MA spidroin from *Araneus diadematus*.[265] Spidroins appear overall as a very attractive raw material in the biomedical field, especially if biocompatibility capacities are accompanied with a mechanical resistance that competes with natural silk or that surpasses other man-made materials.

4.2 BIODEGRADABILITY

Silk can be considered as a durable material in a diversity of environments[245] but biodegradability may be a required for some applications. The interest in that property depends on the use envisioned. Although biodegradability is an asset for environmental reasons at the end of the product's life cycle, a slow degradation of proteins, especially if uncontrolled, may represent a concern for many material usages, for example in the case where long-term structuring/reinforcing function is required.[245] In the case of implants formed *in vivo* by tissue regeneration, the material should be *bioresorbable*, i.e. the scaffold should exhibit a slow or controlled degradability *in situ* as it provides support to cell development and eventually has to be substituted by the newly formed tissue.[248,249,266] Resorbability may also be necessary for suture purposes. Such surgery or repairing filament has however to resist over a reasonable period of time and the resorption rate has to be long enough, especially in the case where fatigue is critical such as for tendon repair.[267] It is thus necessary to control the rate of degradation/resorption.

Evidence suggests that natural silk fibers are susceptible to proteolytic degradation over long periods both *in vivo* and *in vitro*.[249] The phenomenon results in a progressive decrease in the strength of the fiber.[266,268,269] *In vivo*, this occurs within one year for silk fibroin while the material shape can be difficult to identify after two years of implantation.[246] The dragline and Flag silks undergo a slow degradation in a benign environment although a rise in mechanical performances has been noticed during the first year of aging.[268] The rate of degradation varies with the type of silk.[266,268] It is affected by various parameters such as molecular weight, crystallinity, secondary structure (especially β-sheet) and potential points of protease cleavage.[249,270] Furthermore, degradation has been shown to depend on hydrophilic interactions, i.e. on protein organization in the material, especially the alternations of crystal-amorphous nanodomains.[271] It has been found that enzymatic degradation of β-sheets in *B. mori* silk fibroin results in the formation of soluble silk fragments and nanofibrils and then in nanofilaments of ~ 2 nm thick and ~ 160 nm long, that exhibit no cell toxicity *in vitro*.[272]

In the case where biodegradability has to be totally inhibited, several strategies can be envisaged depending whether they are suitable for the intended applications:[245] the silk fiber can be coated with a protective layer, it can be embedded in a specific matrix, it can be infiltrated with a preservative substance (that may alter the structure) or it has to be restrictively used in a dry or abiotic environment.[245]

5. Applications

5.1 Potential advantages of spider silk-based materials

The main motivations to use spidroins aims at taking advantage on their numerous natural properties such as:

- their ability to form hierarchical, nanocomposite materials;
- their superior mechanical (mainly tensile but also torsional and humidity-responsive) features;
- their lightness;
- the fact that the raw material can be obtained from renewable resources;
- Biocompatibility (biosafety and biofunctionality);
- Biodegradation;
- the ability to produce customized primary structures for multi- or novel functionality;
- the low footprint spinning or processing procedures from a chemical and energetic point of view;
- the ability to produce materials with various shapes, states and textures such as sponges, gels, foams, emulsions, macro- or micro-spheres (beads, capsules), nanoparticles, 3D microperiodic lattices, films, 2D mats and meshes, micro- or nano-filaments,[8]
  thanks to different (or a combination of) methods such as:
  - gelation (induced by heat, salt, sonication, solvent, pH, high pressure CO_2, annealing, chemical cross-linking or electrical stimulation) or precipitation,
  - casting,
  - drying (evaporation, sublimation),
  - wet-spinning, electrospinning, and/or
  - patterning (phase separation (emulsification, salting-out, coacervation), spray-drying, direct "ink printing" (Figure 12), nano-imprinting).

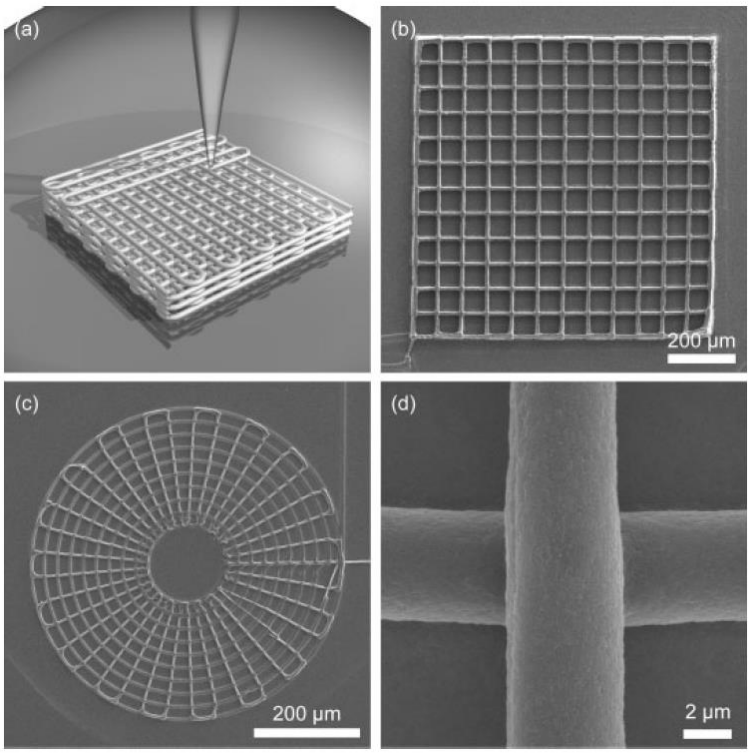

Figure 12. (a) Schematic representation of the 3D direct ink writing principle, a patterning method, using a silk fibroin solution intended to the design of different microperiodic scaffolds for various applications, especially in biomedicine. The ink consisted of a silk fibroin solution from *B. mori* deposited in layers through a fine deposition nozzle to produce under mild ambient conditions a 3D array of filaments of 5 µm diameter. Typical 3D structures obtained: (b) square lattice and (c) circular web. (d) Magnified image of direct write silk fiber. Permission required [69].

Spider silk-based materials may however have some drawbacks or limitations:

- Sensitivity to humidity (see below);
- Biodegradation;
- Relatively low temperature resistance.[245]
- Ecological footprint or ethical concerns of production of the raw material;

Benefits and shortcomings may depend on the chosen application and requirements. Many of these specifications will be determined by the hierarchical organization of the material that in turn will be dictated by the protein sequence and by the process by which it will be structured. Thus, the design of a protein intended to prepare a specific biomaterial should simultaneously incorporate various, and maybe antagonist, constraints such as appropriate mechanical, optical, biocompatibility and biodegradability

properties, while taking into account at the same time the ecological footprint of its life cycle. These requirements should be considered from the beginning of the design process of the material.

After about 50 years of investigation in silk research, the applications of silk seems endless today. The future will determine those that will be the most interesting from an environemental, performance and ecomic point of view. *B. mori* silk has been the subject of the majority and the most advanced applications to date. The sections below will therefore also report laboratory researches based on silkworm silks to illustrate avenues that may potentially be explored with spider silk. Since the range of applications is quite broad, the following selected examples are given to illustrate some of the future possible avenues.

5.2 Applications as high-tech thread

Since the mechanical performances of natural spider fibers are remarkable and diverse, and for some of them superior to those of industrial fibers, many works are intended to produce "strong" fibers as seen above. Several applications may thus probably be devoted to the production of various specialty threads or to the incorporation of synthetic spider silk fibers as a component of such high-tech filaments. Among the multiple potential future uses, synthetic spider silk is anticipated to constitute parachute cord, bullet-proof vest, specialty rope (for example in the sport or textile industry), fishing nets or reinforcement constituent in polymer matrices, for instance in the aviation industry, etc.[273,274] Tensile performances may be improved by infiltrating the biomaterial with inorganic compounds such as metals.[275] Torsional particularities of the dragline thread, in conjunction with its tensile characteristics, might also find other future applications, for instance in rock climbing.

5.3 Textile

The silk produced by the mulberry silkworm *B. mori* has a long tradition in the textile industry. It is highly appreciated due to its mechanical strength, luster, drapability and smooth feel texture. It exhibits however several drawbacks such as wrinkling, degradation, low wet resiliency and UV-induced yellowing.[13,276-278] Although *B. mori* yarn can be improved at various levels, spider silk biofibers could also solve or reduce some of these concerns while providing higher mechanical resistance. Spider silk materials might also arise interest in the textile sector to develop fibers with added value, including multifunctionality or new properties such as dyeing (colored, luminescent fibers),[279] anti-aging, anti-bacterial and self-cleaning capabilities.[13,277]

5.4 Biomedicine

Since the supply of the raw material remains a fundamental problem, products based on spider silk may not be viable economically in the short term. At least, the cost of production may be anticipated to be high, especially because large-scale production may be difficult to achieve and due to the multi-step purification procedures required. Therefore, specialized applications, particularly in the biomedical field, is probably the most suitable sector for the first future applications of spider silk-based materials where particularly load-resistant fibers are required.

Spider silk appears then as a promising tool with a broad range of applications as medical devices.[13,15,24,237] Silk would then be advantageously used as drug delivery systems,[27,29,280-282] scaffolds for tissue regeneration and implants (nerve[254], blood vessel,[283] tendons,[284,285] ligaments,[286] cartilage,[260,287] bone,[257,264,288,289] skin[289] or other extracellular matrices (fibroblasts)[253,258]), wound repair (suture,[267] wound dressing[290-292]) and surgery (eye, lip, oral and neuronal), especially due to its biofunctionality qualities and if its biosafety security is unambiguously established.

Like for all proteins, several strategies may be implemented to improve or customize the properties or to provide new or multiple functionality of the final material. These objectives may be achieved by chemically functionalizing the protein sequence (for instance with RGD motifs or other chemical groups),[28,293] by producing hybrid (fusion or chimeric) proteins,[28,237,294] and/or by forming composites made of protein mixtures,[28,288,295,296] protein and other macromolecules,[27,297] or protein-inorganic compound mixtures.[238,298]

5.5 Optics and electronics

Silk can also exhibit interesting optical, photonics and electronics responses.[24,237] Silkworm silk has fruitfully been used in this area of research and it may be anticipated that spider silk-related proteins could also make significant contributions. Thanks to surface nanopatterning, optically transparent fibroin materials have been developed to form bioactive devices for optical applications such as diffraction gratings, pattern generators and lenses,[12] in particular via the control of β-sheet content. The "direct ink writing" method has been used to produce optical waveguides, which offers new possibilities for creating biophotonic elements that can be readily doped or functionalized with biologically active agents.[299]

Furthermore, due to the biocompatible characteristics of silk-based materials, devices may be transferred in the human body for new advanced biomedical applications. Some optical implantable devices may find applications for diagnosis and/or cure. For instance, the ability of fibroin to form porous structures has been exploited to create implantable photonic crystals, namely inverse opals.[300] The optical properties of these devices are determined by a periodic modulation of their refractive index in one or more dimensions. In this case, the modulation is imparted by the periodic distance between pores and by the dielectric constant of the filling material.[300] Such a system may be useful for biosensing *in situ* but it may even show promises for advanced tissue regrowth since inverse opals could allow for both optical monitoring and cell proliferation.[301] Alternately, these photonic crystals could be used as implantable bio-therapeutic devices to eliminate pathological tissues or cells by hyperthermal effects (localized heat induced by interactions with IR radiation).[301]

Recently, the pristine spider dragline silk fiber has been tested as an optical fiber.[136] In this experiment, light has been transmitted in a straight as well as in a bent filament. The fiber has also been integrated in a photonic chip made of polymer microstructures fabricated by UV-lithography, leading to an efficient micro-optical coupling between silk and synthetic optical structures.[136] Another example is provided by a silicon nanomembrane electronic device that has been integrated on a silk substrate and then implanted *in vivo*. This system constitutes an implantable electronic device that is bioresorbable and adaptable to non-planar organ or environment.[302,303] This dissolvable and implantable bio-integrated electronics

would be compatible with active electronics and optoelectronics[303] thus offering a large range of applications for medical monitoring and therapeutics. In another study, *B. mori* silk has been used to design wireless passive food sensors that consist of a microfabricated antenna or an array of antennas/resonators made of gold deposited on a silk fibroin substrate. These radio frequency identification (RFID)-like silk tag sensors are flexible and can adopt the non-planar shape of fruits or vegetables.[304] The system relies on the fact that the response of these antennas is affected by the dielectric properties of the compound to be probed. Their resonant responses were successfully tested during the ripening process to assess the potential for monitoring changes due to food spoilage.[304]

5.6 SYNTHETIC MUSCLES AND ACTUATORS

As seen above, silk can be very sensitive to water and humidity. Upon exposure to liquid water or water vapor, silk can shrink longitudinally and swell (Figure 13), while the plasticizing effect of water strongly affects its mechanical behavior. Dragline silk for example undergoes a decrease in Young modulus and an increase in breaking strain in wetter environments.[124,305] This sensitivity to water may be an advantage or a shortcoming depending on the application. Changes in material characteristics in a moisture-dependent manner may make the use of spidroins totally prohibitive.[245] This is particularly true in the biomedical area, as water-induced contraction phenomena for instance are usually removed from materials.[244 249]

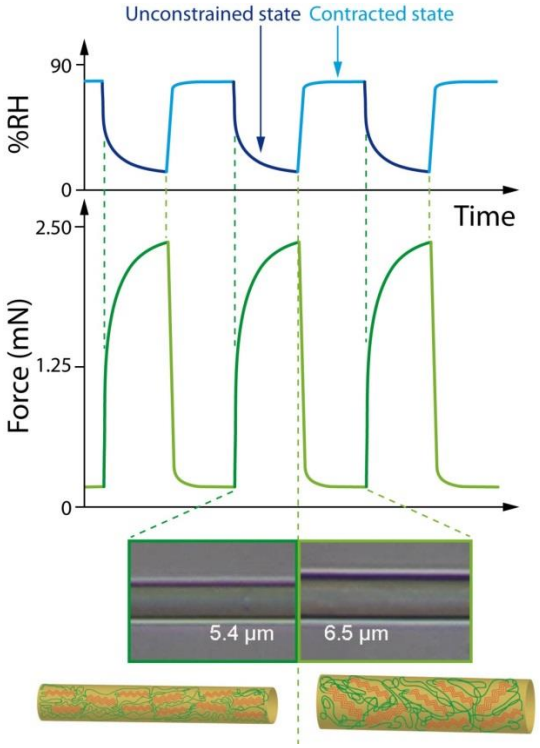

Figure 13. Use of spider dragline silk as an actuator. The fibre is submitted to cyclic relative humidity (RH) variations (blue curve) to generate reversible contraction of the fiber which induce physical forces (green curve) that can be used for artificial muscle purposes. (Adapted from Ignarsson et al. *J Exp Biol* **212** (2009) 1990). The microscopic images show the comparison of the diameter of unconstrained and

contracted fibres. Schematics below show corresponding representations of the semicrystalline structure (red: β-sheets; green: amorphous phase).

However, the fact that the maximally supercontracted state represents a ground state for silk may allow tailoring mechanical behavior of synthetic fibers. As a matter of fact, it has been shown that the whole range of tensile properties exhibited by natural fibers can be reproduced by following a given sequence of drawing steps in water.[128] Supercontraction may also lead to further applications such as artificial muscles. It is indeed possible to generate humidity-induced cyclic contractions/relaxations of dragline silk, thus allowing it to act as a biological actuator. Dragline contraction is unique as it can induce a mechanical work fifty times larger than the equivalent mass of human muscle.[306] The control of supercontraction and the production of twisted threads[307] may eventually lead in the development of temperature- and/or humidity-driven high-performance robots and micro-machines, new sensors, smart textiles or green energy production devices.[306-308]

Conclusion and future challenges

Nature has provided us with very few examples of materials manufactured at rates comparable to Human fabrication timescales. Silk is one rare natural model from which scientist can take inspiration. While synthetic materials are generally submitted to a trade-off between strength and extensibility, silk, like other natural materials,[119] appears as an astonishing compromise between these apparently antagonistic parameters. Moreover, silk is made of relatively simple constituants, i.e. spidroins, whose mechanical-induced assembly response is subtly adjusted by the soft aqueous environment of the spinning gland.

Silk fibers have evolved during 380 millions years to contribute to the feeding, reproduction and survival success of spiders. Most often, silks are multifunctional so that their properties have to fulfill various mechanical constraints with the lowest metabolic cost for the animal. The MA fiber of Orbiculariae for example is a versatile thread that has to be efficient as lifeline and frame and attachement of the web.

Then, the spidroin sequence appears as a striking trade-off between the requirement of being soluble and stable for a relative long term in the gland while having a propensity to rapidly and adequately assemble into an insoluble material when submitted to mechanical stresses. Spidroins are in fact programmed to form a specific structure under precise chemical conditions when submitted to the stresses induced by spiders. This, however, does not exclude plasticity of silk properties since they vary, but still remain functionnal, depending on diet, external conditions (humidity, temperature), weaving conditions (spinning rate).

Spidroin sequence and spinning processing have then evolved to provide the appropriate structure (i.e. the appropriate properties) in spiders' ecological context. For the MA silk, this lead to optimal structural and processing features, as nicely underlined by earlier and more recent researches:

hierarchical/composite architecture (optimization of the arrangement at the nano-, meso and microscopic scales), spidroin sequence design, dope rheological properties and dope chemical composition.

Spidroin sequences in particular incorporate various characteristics that are important for the fibre structure and spinning such as molecular weight, amino acid composition (particularly Ala, Gly, Pro), sequence pattern (modular structure and NR terminal domains), flexibility, hydrophobicity/hydrophilicity, secondary structure propensity and β-aggregation propensity. Every detail of the primary structure and physicochemical environment seems important to achieve the final structure of the fiber.

Such a complexity is obviously difficult ro reproduce in vitro, which explains why it is so difficult to mimic silk. Scientists have to overcome several technological challenges regarding the processing of spidroin-based materials and its efficiency as well as regarding the control of the final structures and properties. One of the main difficulty is to perfectly mimic the spinning process of spiders, especially the use of water as solvent and achieving sufficiently high protein concentrations in solution. Among various emthods, microfluidic techniques may offer the most appropriate opportunity to manufacture synthetic silk with a processing that resembles the natural spinning process as close as possible.

The understanding of the spinning process, fiber structure and its impact on silk properties has made notable progresses, especially for MA silk. Computer simulations now allows mesoscale approaches[309] and the study of the conversion of the spidroin molecules in solution into a fiber.[148] Still, new experimental and modelisation breakthroughs are required to fully understand the complexity and subtleties of the relation between sequence, structure and properties. To fully take advantage of silk and widen our range of models, it would also be very useful to deepen our understanding of the other types of silk, in particular those that do not possess a modular sequence pattern such as Ac silk.

Even for the MA silk, among the structural key features that explain the outstanding properties of silk (primary and secondary structures, nanoscale aspects (nanoconfinment, crystal size), microstructure (fibrillar, skin/core)), the contribution of each one remains unclear. Modelisations that integrate all hierarchical length scales are required to fully understand silk threads. The relation between rheological behavior of the dope and its molecular and microscopic structure is also still fragmentary. Such progesses would help in the production of synthetic spidroin analogs that may form appropriate hierarchical structures (i.e. with appropriate properties). Furthermore, advances made in the silk domain may have more general benefits to the design of synthetic materials.

Despite difficulties, due do its mechanical properties and the potential to produce ecofriendly materials, spider silk and its synthetic derivatives constitute probably one of the most promising avenue of research in material science for diverse advanced, multifunctional applications (mechanically strong and/or stretchable, functionalizable, biocompatible, biodegradable biomaterials). Gradual progresses in the fabrication of regenerated fiber suggest that scientists will succeed in making silk materials with improved properties and lower ecological footprint. Potential advantages of using silk are summarized in Figure 14.

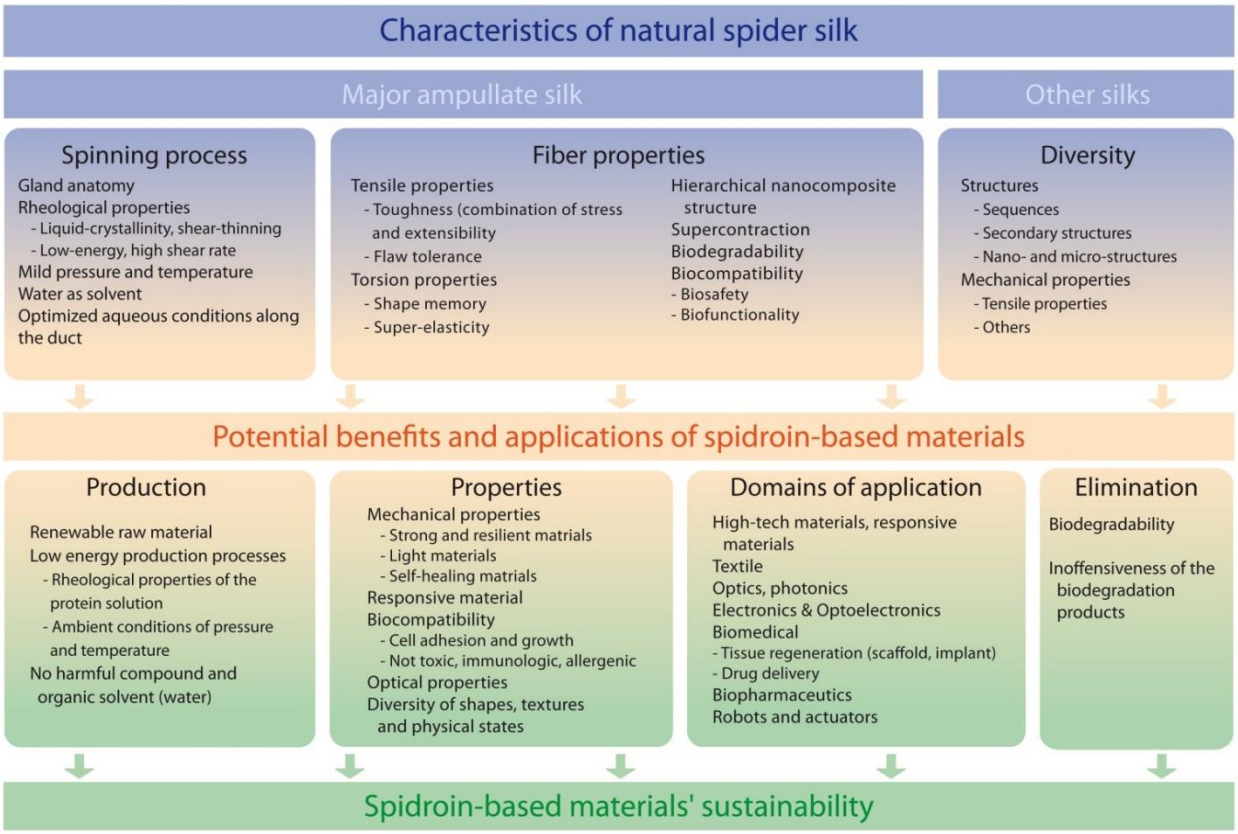

Figure 14. Potential benefits of natural spider silk for spidroin-based materials. The emphasis is put on MA silk since it is the most studied.

It seems that in the near future the main advances will principally be achieved with *B. mori* fibroin because it it much more easily available than spider silk and can already lead to remarkable functional materials. This is anyway a good model to develop efficient methods for the fabrication of silk materials comparable to natural ones and these tools may subsequently be applied to spidroins. Other strategies may also be envisaged. Since the fabrication of purely “spidroinaceous” materials that are as tough as the natural fiber remains difficult, the design of hybrid silk-based materials reinforced with nanoparticles or carbon nanotubes has proved to provide materials with promising mechanics (Table 2) and may be an attractive (temporary?) alternative. Due to the actual and short-term future high economical cost of silk-based materials, it is expected that silk may first find applications in the biomedical field.

From a “green” perspective, in addition of exhibiting desired properties, silk-based products will have to minimize their environmental impact. Then, besides their green processing, their entire life cycle assessment will have to be considered. This includes environemental aspects such as product durability and repair, production of (recombinant) spidroins, processing, transportation, use, maintenance, and recycling, degradation into innocuous compounds or ultimate disposal at the end of its useful lifespan. This is particularly true for the production of recombinant spidroin by biotechnology that may have various environemental impacts in term of energy and water consumption depending on the host chosen (bacteria, plant, mammals). In this respect, if materials with exceptional mechanics are not

indispensable, *B. mori* silk or byproduct proteins (originating from industrial wastes) such as bovine β-lactoglobulin, soy proteins, wheat gluten, feather keratin, mussel byssus, cellulose or collagen, may represent attractive alternatives to spider silk since they are available in large quantities and may be advantageously used from an environmental point of view.[190]

Besides silk, it is recognized that materials science will play a pivotal role in sustainable development (SD).[310] Efforts devoted to the reduction of the ecological footprint of materials are crucial and obviously necessary. They lie within a more general framework aiming at reducing the ecological footprint of technology. Although, this objective is essential for societies, it is insufficient to make them sustainable as SD requires first of all transformations of the economy, social behaviors and governance. This is why materials science should be integrated into a broader society's view. For example, besides environmental issues, the production of recombinant spidroins may have beneficial or detrimental social repercussions. The expression of protein in plants for instance require lands dedicated to agriculture which may represent opportunities for farmers or may come into conflict with the use of territories for food production or exacerbate the pression on natural lands (forests, grassland, etc.). Such considerations of course go beyond materials science itself and require a broad, multidisciplinary perspective. This view, aiming at making society activities sustainable, should prompt materials scientists to take part in this complex and stimulating societal project.

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