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MINI-REVIEW

To spin or not to spin: spider silk fibers and more

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Abstract Spider silk fibers have a sophisticated hierarchical structure composed of proteins with highly repetitive sequences. Their extraordinary mechanical properties, defined by a unique combination of strength and extensibility, are superior to most man-made fibers. Therefore, spider silk has fascinated mankind for thousands of years. However, due to their aggressive territorial behavior, farming of spiders is not feasible on a large scale. For this reason, biotechnological approaches were recently developed for the production of recombinant spider silk proteins. These recombinant proteins can be assembled into a variety of morphologies with a great range of properties for technical and medical applications. Here, the different approaches of biotechnological production and the advances in material processing toward various applications will be reviewed.

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Introduction

Spider silks represent a class of fibers with a unique combination of strength and flexibility which leads to an outstanding toughness (Gosline et al. 1999). In comparison to one of the strongest man-made fibers, Kevlar, spider silk can absorb three times more energy before breaking (Roemer and Scheibel 2007). Therefore, it is not surprising that ancient Australian aborigines and New Guinean natives utilized spider silk as fishing lines, fishing nets, head gear, and bags (Lewis 1996). Further, until World War II, spider silk was used for crosshairs in optical devices like microscopes, telescopes, and guns because of its extremely small diameters (thickness of 1/40 of a human hair) (Gerritsen 2002; Lewis 1996). By using cobwebs to stanch bleeding wounds, the ancient Greeks unknowingly observed further extraordinary characteristics of this material, like high biocompatibility and low immunogenicity (Altman et al. 2003; Gerritsen 2002; Vollrath et al. 2002). However, the first scientific studies to unravel its biomedical properties were not started until 1710, when it was shown that a spider's web is able to stop bleeding in human wounds and also supports the wound healing (Bon 1710). Two centuries later, Otto G. T. Kiliani investigated spider silk as suture material for surgery (Kiliani 1901).

As illustrated by the long history of spider silk use, the outstanding properties of natural spider silk have been well-known for a long time; however, scientifically, the material attained intensive interest of researchers only in the last decades. The combination of mechanical performance, biodegradability, and ambient processing conditions of the underlying proteins makes spider silk a highly desirable material for

applications from biomaterials to high-performance industrial fibers (Rising 2014; Vollrath and Knight 2001).

Spider silk structure

Female orb weaving spiders can produce up to six different types of silk, with each one produced in a specialized gland that provides the name of the corresponding silk type (Fig. 1). Every silk type has to fulfill a certain task either in the structure of the web, the protection of the offspring, or the wrapping of prey. Additionally, a silk-like glue, produced in a seventh gland, is deposited on the web for prey capture. The most frequently investigated silk type is the dragline silk, used to build frame and radii of an orb web. It also is used as the lifeline of the spider and is therefore easy to obtain by forced silking (Andersen 1970; Blackledge and Hayashi 2006; Denny 1976; Heim et al. 2009; Vollrath 2000). Similar to many biological materials, the outstanding (mechanical) performance of spider silk is based on its hierarchical structure (Brown et al. 2012; Keten and Buehler 2008; Munch et al. 2008; Smith and Scheibel 2013; Sponner et al. 2007). Dragline spider silk fibers exhibit a core-shell structure with proteinaceous fibrils in the core and a three-layered shell of minor ampullate (Mi) silk, glycoproteins, and lipids. While the lipid part of the shell is only loosely attached to the core and does not substantially contribute to the mechanical performance of the fiber, the glyco-layer adheres directly and is a mediator between the fiber and its environment (Sponner et al. 2007). In this context, the shell is thought to be relevant for protection against environmental damage and microbes (Sponner et al. 2007). However, the determinant of the extraordinary mechanical characteristics of spider silk is the proteins which form the core of the fiber. The protein core of dragline silk is composed of two classes of spider silk proteins (spidroins): the highly ordered, hydrophobic spidroin I (Sp1), poor in proline residues, and the less ordered, hydrophilic, proline-rich spidroin II (Sp2), each with a molecular mass of

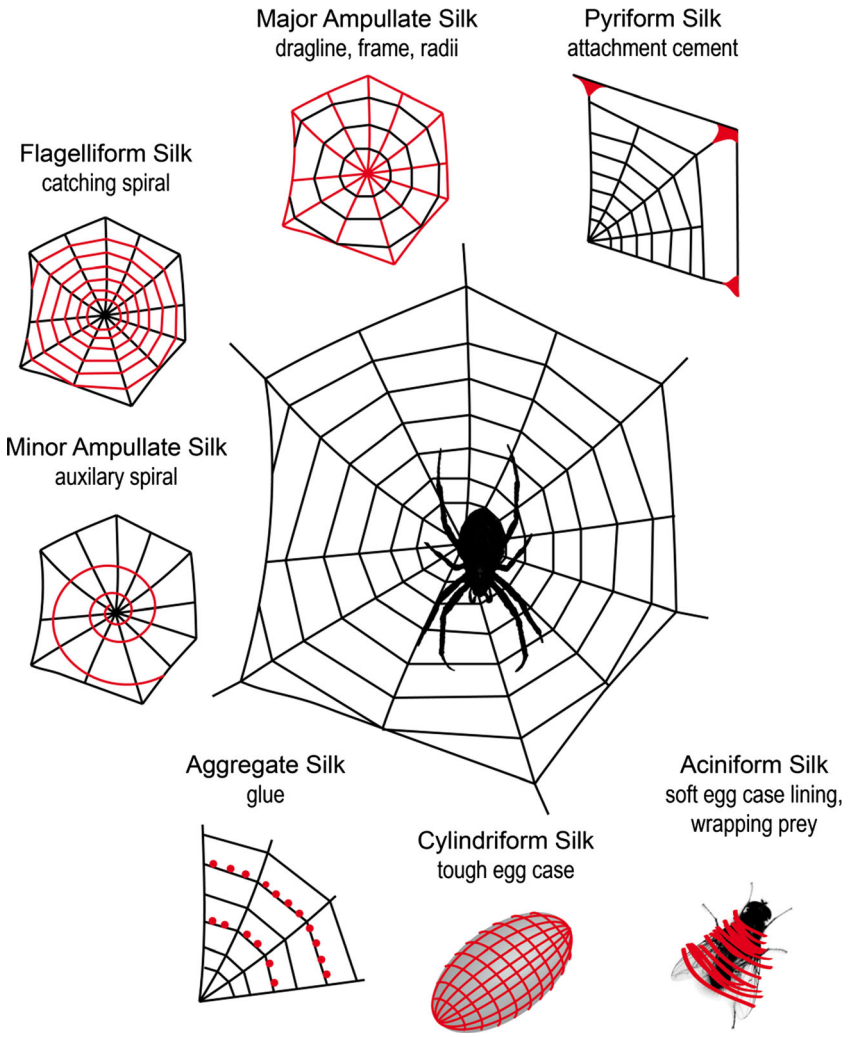

Fig. 1 Schematic overview of the different types of silk produced by female orb weaving spiders (Araneae); each silk type (highlighted in *red*) is tailored for a specific purpose as depicted

around 300 kDa (Heim et al. 2009; Xu and Lewis 1990) (Ayoub et al. 2007; Hinman and Lewis 1992; Xu and Lewis 1990). As they originate from the major ampullate gland, these proteins are also called major ampullate spidroins (MaSp). All MaSps comprise a highly repetitive core domain (up to 100 repeats of highly conserved sequence motifs, with 40 to 200 amino acids each) flanked by short (around 100–150 amino acids each) nonrepetitive (NR) terminal domains (Fig. 2). Upon fiber assembly, the gain and arrangement of secondary structure elements of the spidroins is responsible for the extraordinary mechanical properties of the fiber. Poly-alanine stretches fold into β-sheets, forming hydrophobic crystallites responsible for a high tensile strength (Kummerlen et al. 1996; Lewis 1992; Simmons et al. 1996); 3_1-helices formed by hydrophilic glycine-rich regions (GGX-motif, where X represents tyrosine, leucine, glutamine) are reflecting the elastic part (Kummerlen et al. 1996); and type II β-turns made of proline-rich GPG motifs are important for the reversible extensibility of a spider silk fiber (Hinman and Lewis 1992). While the latter sequence motif is only present in MaSp2, the first two motifs are ubiquitous (Ayoub et al. 2007; Hayashi et al. 1999; Hinman et al. 2000; Hinman and Lewis 1992; van Beek et al. 2002). All these motifs are repeated several dozen times within a single spidroin core domain. The nonrepetitive terminal motifs which flank the core domain have an α-helical secondary structure arranged in a five-helix bundle. These domains are responsible for controlling the storage of the spidroins at high concentrations in the spinning duct (Motriuk-Smith et al. 2005), and they also have an important function during the initiation of fiber formation upon their controlled dimerization and structural arrangement (Challis et al. 2006; Eisoldt et al. 2010, 2011; Hagn et al. 2010, 2011; Hedhammar et al. 2008; Heidebrecht et al. 2015; Huemmerich et al. 2004b; Rising et al. 2006)

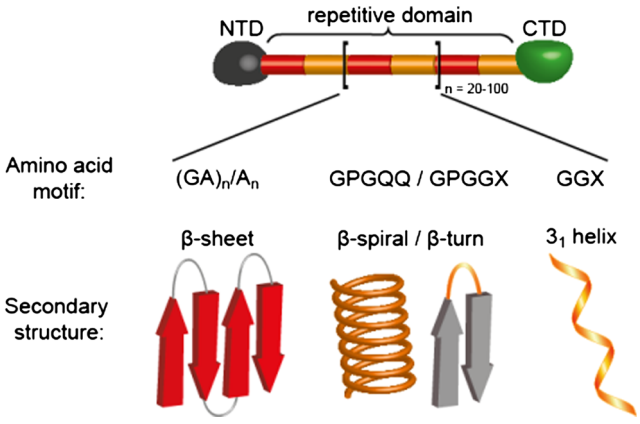

Fig. 2 Schematic structure of major ampullate spidroins including recurring amino acid motifs and the corresponding secondary structure. X: predominantly tyrosine, leucine, glutamine, alanine and serine residues. *NTD* amino-terminal domain, *CTD* carboxy-terminal domain

Biotechnological production of recombinant spider silk proteins

Unfortunately, it is not possible to produce large quantities of spider silk for applications by farming. This is due to the territorial and cannibalistic behavior and lower quality as well as quantity of silk produced by captive spiders (Craig et al. 2000; Fox 1975; Madsen et al. 1999; Vollrath and Knight 1999). Therefore, biotechnological production of the underlying spidroins was pursued to enable applications for spider silks.

Recombinant spidroin production has been conducted using a range of organisms including bacteria (Teule et al. 2009), tobacco plants (Menassa et al. 2004), yeast (Fahnestock and Bedzyk 1997), silk worms (Teule et al. 2012), goats (Steinkraus et al. 2012), insect cells (Huemmerich et al. 2004b), and mammalian cells (Lazaris et al. 2002). Each of these host systems has advantages and disadvantages. To begin with, short fragments of unmodified spider silk genes were expressed in a variety of hosts. It turned out that spider silk genes were unstable or the mRNA folded into undesirable secondary structures. Further, rearrangements, translation pauses, and problems with PCR amplification arose due to the highly repetitive character of the genes and the infidelity of template realignment during primer annealing (Fahnestock and Irwin 1997; Fahnestock et al. 2000). Additionally, host-derived differences in codon usage, problems with expression of repetitive sequences in various hosts, and insufficient Gly- and Ala-tRNA pools led to only limited success concerning the recombinant production of natural spider silk proteins.

To overcome these hurdles, several synthetic genes were designed encoding proteins that resemble the key features of the natural spider silk proteins. Since the gram-negative enterobacterium *Escherichia coli* is relatively simple, has a well-known genetic composition, and has the capability of fast, high-density cultivation, recombinant protein expression in *E. coli* allows for inexpensive, large-scale production (Sørensen and Mortensen 2005). Likewise, several approaches of recombinant spider silk-like protein production were successful in *E. coli* (for an overview, see Heidebrecht and Scheibel 2013).

In addition to *E coli*, yeast or insect cells have been used to express spider silk constructs with the advantage of the latter of being genetically more closely related to spiders. However, the spidroins produced in these systems showed a quite low solubility (Heim et al. 2009; Huemmerich et al. 2004b). Other hosts such as plants and mammalian cells have been used, too, but showed mostly low expression levels (Barr et al. 2004; Hauptmann et al. 2013; Lazaris et al. 2002).

Finally, transgenic animals were tested as hosts to produce recombinant spidroins in secreted body fluids. The presumed advantage of this approach would be the ease of purification

upon secretion into the milk or urine of the respective animal (Heim et al. 2009; Karatzas et al. 1999). However, it turned out that the purification was more difficult than thought due to contamination with animal-based secreted proteins. Given the fact that the generation of transgenic animals is far more complex and time consuming than that of bacteria or yeast, this approach has been rarely used in the past (Heim et al. 2009; Xu et al. 2007). For example, recombinant spider silk-EGFP fusion proteins were produced using BmN cells and larvae of silkworms as a host organism, but the protein yield was low due to the insolubility of the recombinant spider silk proteins (Zhang et al. 2008). In a more successful approach, chimeric proteins containing sequences of spider silk proteins and silkworm fibroin were designed, including either a H-chain promoter (Kuwana et al. 2014; Teule et al. 2012; Zhu et al. 2010) or a sericin promoter (Wen et al. 2010) locating the chimeric silkworm/spider silk proteins in the core or the sericin shell of the fiber. In both cases, silkworms spun fibers with mechanical properties exceeding that of silkworm silk, but they did not reach the properties of natural spider silk (Teule et al. 2012; Wen et al. 2010). Production of designed short spider silk proteins (50 kDa) resembling MaSp1 and MaSp2 of *Nephila clavipes* in goat milk was also successful, while expression of their partial complementary DNA (cDNA) in transgenic mice was not possible likely due to errors in protein synthesis (Perez-Rigueiro et al. 2011; Xu et al. 2007).

Based on the experience throughout the last three decades, *E. coli* has been established as the host system of choice, given the balance of quality of the silk produced with the scalability of the approach.

"To spin": artificial spider silk fibers

Due to the abovementioned, outstanding mechanical and biomedical properties of spider silk fibers, great efforts have been made to employ these fibers in different technical as well as biomedical applications. For instance, functional recovery of nerve defects was successfully performed in rats and sheep by using natural spider silk fibers as a guiding material (Allmeling et al. 2008; Radtke et al. 2011). Further, native spider dragline silk, directly woven onto steel frames, was used as a matrix for three-dimensional skin cell culture (Wendt et al. 2011). Since natural spider silk fibers are not available at large scale as mentioned above (see section "Biotechnological production of recombinant spider silk proteins"), different approaches have been tested to produce artificial spider silk fibers during the last two decades, which will be discussed in greater detail below.

The natural spinning process

In order to successfully establish a man-made spider silk spinning process, it is at first necessary to understand the natural one. Natural spider silk fiber spinning is a highly complex process involving a number of parameters in a highly regulated environment as exemplarily demonstrated in Fig. 3 for the assembly of major ampullate spidroins. Epithelial cells covering the tail and the ampulla of the major ampullate silk gland produce the spidroins and secrete them into the lumen. There, the spidroins are stored in a soluble state at high concentrations (up to 50 % (*w*/*v*)) in the presence of sodium and chloride ions. Analysis of major ampullate silk glands by polarized microscopy revealed a liquid-crystal behavior of the spinning dope (Knight and Vollrath 1999; Viney 1997), whereas in vitro experiments showed micellar-like structures both of which are not mutually exclusive (Eisoldt et al. 2010; Exler et al. 2007; Heidebrecht et al. 2015). The combination of the presence of chaotropic ions (stabilizing soluble protein structures) and a pre-assembly of the spidroins enables their storage at concentrations as found in the ampulla of the spinning gland. From the ampulla, the spinning dope passes into an S-shaped tapered duct, which is lined by a cuticular intima layer. In addition to supporting the duct and protecting the epithelial cells, this layer is hypothesized to function as a hollow fiber dialysis membrane, enabling the dehydration of the spinning dope (Vollrath and Knight 1999). During traveling of the spinning dope through the spinning duct, sodium and chloride ions are replaced by the more kosmotropic potassium and phosphate ions inducing salting-out of the proteins (Knight and Vollrath 2001; Papadopoulos et al. 2007). Additionally, acidification (from pH 7.2 to 5.7; Kronqvist et al. 2014) takes place triggered by carbonic anhydrase (Andersson et al. 2014), which has a contrary structural effect on the terminal domains. Upon acidification, glutamic acid residues of the amino-terminal domain are sequentially protonated, leading to structural rearrangements of the domain enabling dimerization in an antiparallel manner (Rising and Johansson 2015). In contrary to the stabilizing effect of the pH reduction on the amino-terminal domain, the carboxy-terminal one is destabilized upon acidification. In addition to the pH-induced destabilization, the presence of phosphate ions initiates the exposition of hydrophobic areas within the C-terminal domain initiating the parallel alignment of the associated two core domains (Eisoldt et al. 2010, 2012; Hagn et al. 2010). Based on the parallel (carboxy-terminal domains) and antiparallel (amino-terminal domains) orientation of the terminal domains, an endless spidroin network is achieved. Finally, water resorption via the cuticular intima layer and shear stress, resulting from the tapering of the spinning duct and the pulling of the fiber from the spider's abdomen, lead to the final alignment of the spidroins followed by solidification of the fiber (Fig. 3) (Hagn et al. 2011; Hardy et al. 2008).

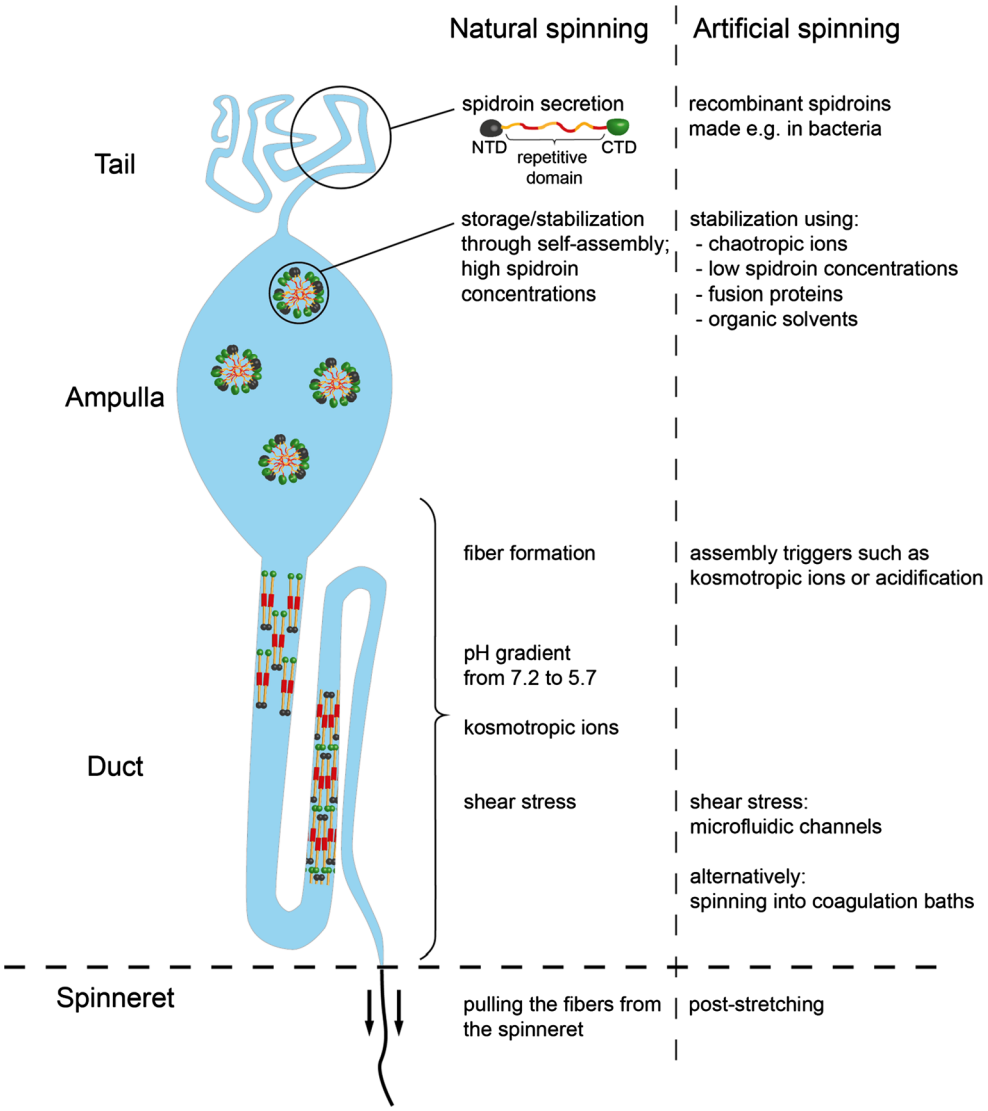

Fig. 3 Overview of natural and artificial spinning processes

Artificial fiber spinning

Commonly used artificial spinning processes are not like the natural silk spinning one. Typical processes out of solution are wet spinning, dry spinning, and electrospinning. In wet spinning, a polymer solution is extruded into a coagulation bath, where the polymer precipitates and the fibers are formed. For dry spinning and electrospinning, the polymers are solvated in an organic solvent and extruded into the air. Whereas fiber formation in dry spinning relies solely on the fast evaporation of the organic solvent, in electrospinning, the polymer solution is extruded into an electrostatic field. This field yields repulsive forces in the extruded solution, leading to eruption of a thin jet that is stretched toward the collector (i.e., counter electrode); as the solvent evaporates, a solid fiber is formed (Greiner and Wendorff 2007; Smit et al. 2005). This fiber is randomly deposited onto the collector, which results in a nonwoven mat (Teo and Ramakrishna 2006). In theory, wet spinning, dry spinning, and electrospinning are suitable methods for spider silk fiber spinning, since organic as well as aqueous spinning dopes can be used. In practice, dry spinning has been shown to be so far not suitable for silk fiber production, since spinning a silk fiber out of an organic solution results in mechanically unstable fibers (unpublished results), while dry spinning from an aqueous solution could not be achieved since this spinning technique relies on a highly volatile solvent for fast fiber formation. Therefore, so far, only wet spinning and electrospinning have been successfully employed for producing artificial spider silk fibers.

Dope preparation

The first step toward the production of artificial spider silk fibers is to solve the spidroins. Therefore, often an organic solvent is used exhibiting strong hydrogen bonding properties in order to guarantee good solvent-protein interactions. A

disadvantage, especially for biomedical applications, of organic spinning solutions is their putative toxicity. However, a high spidroin solubility enables the production of highly concentrated spinning dopes, which simplifies fiber formation (Um et al. 2004). With the objective of high protein solubility, many research groups have used the organic solvent 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP). In HFIP, spidroin concentrations ranging from 10 to 30 % (*w*/*v*) can easily be achieved (Adrianos et al. 2013; An et al. 2011; Brooks et al. 2008; Lin et al. 2013; Teule et al. 2007; Xia et al. 2010), with the highest reported spidroin content of 45–60 % (*w*/*v*) (Albertson et al. 2014). One advantage of HFIP as solvent for spidroins is its volatility. Therefore, HFIP is commonly used for spinning processes which rely on a fast evaporation of the solvent such as electrospinning (Bini et al. 2006; Lang et al. 2013; Stephens et al. 2005; Wong Po Foo et al. 2006; Zhu et al. 2015). In addition to HFIP, formic acid (FA) has been used as an organic solvent of spidroins (Peng et al. 2009).

Seidel et al. (1998, 2000) dissolved dragline silk of *N. clavipes* in HFIP, produced a film out of the reconstituted spidroins, and then solved this film again in HFIP to a concentration of 2.5 % (*w*/*w*) in order to use it as a spinning dope for wet spinning. Dopes made of reconstituted spidroins did not form fibers in the otherwise commonly used methanol and isopropanol coagulation baths, but only in acetone coagulation baths (Seidel et al. 1998).

At first glance, using an organic solvent to gain solutions with a high protein concentration seems to be beneficial for spinning, but good protein-solvent interactions and, therefore, high protein solubility may also prevent protein assembly. Further, if artificial spider silk fibers are to be used for medical applications like suture materials, health risks caused by toxic solvents have to be avoided. Additionally, organic waste disposal in industrial processes is highly regulated and expensive; thus, the application of organic solvents is not favorable for scale-up processes. In order to avoid organic solvents, three approaches have been used to produce highly concentrated aqueous spidroin solutions: (1) spidroin self-assembly in aqueous buffers (Exler et al. 2007; Grip et al. 2009; Heidebrecht et al. 2015; Stark et al. 2007; Teule et al. 2007), (2) concentration of a diluted aqueous spidroin solution (Arcidiacono et al. 2002; Heidebrecht et al. 2015), and (3) direct solvation at high spidroin concentrations (Bogush et al. 2009; Jones et al. 2015). Protein concentrations typically used for spinning fibers out of aqueous solutions range from 10 to 30 % (*w*/*v*) (Arcidiacono et al. 2002; Bogush et al. 2009; Exler et al. 2007; Heidebrecht et al. 2015; Jones et al. 2015; Lazaris et al. 2002), and the highest concentration achieved so far has been 30 % (*w*/*v*) (Bogush et al. 2009). When spidroins are purified by a precipitation step such as salting-out or lyophilization, high spidroin purities are gained, but the spidroins also have to be resolved afterwards.

Heidebrecht et al. (2015) used the strong denaturant guanidinium thiocyanate for spidroin solvation, followed by its removal using dialysis against a 50-mM Tris/HCl buffer (pH 8.0). Additionally, 100 mM NaCl was added to the dialysis buffer in order to stabilize the spidroins in solution. Subsequent dialysis against a phosphate-containing buffer induced a liquid-liquid phase separation of the spidroin solution into a low-density phase and a self-assembled, high-density phase. Such phosphate-induced self-assembly of spidroins in solution resulted in spidroin concentrations ranging between 9 and 11 % (Heidebrecht et al. 2015). Alternatively, spinning dopes were produced by concentrating the protein solution using either ultrafiltration or dialysis against the hygroscopic polyethylene glycol (PEG) (Arcidiacono et al. 2002; Heidebrecht et al. 2015). In this approach, the spidroin molecules are forced into a highly concentrated solution and they cannot self-assemble. However, these spinning dopes are prone to aggregation and are less stable than self-assembled spinning dopes (Heidebrecht et al. 2015). The third approach to achieve highly concentrated aqueous spinning dopes is the direct solvation of spidroins in a medium suitable for spinning. Jones et al. (2015) added a solution containing 0.1 % propionic acid and 10 mM imidazole to spidroins in a glass vial and used sonication and subsequent heating in a microwave oven until complete spidroin solvation (Jones et al. 2015). The spidroin suspension was heated up to 130 °C for more than 48 h, indicating the high energy input that is needed to directly solve a spidroin at high concentration.

Wet spinning

Extrusion of the spinning dope into monohydric alcohols, such as methanol, ethanol, or isopropanol with the exemption of reconstituted spider silks which have to be spun into acetone as mentioned above, initiates fiber formation through dehydration of the spidroins. This technique results in single fibers with a diameter in the micrometer range. An advantage of wet spinning over other techniques such as electrospinning is the rather “slow” fiber formation, which allows a high degree of alignment of the proteins during spinning. This alignment enables the formation of a structural hierarchy necessary to produce fibers with superior mechanical properties. Wet spinning allows the use of different spinning dopes, ranging from inorganic or aqueous solutions to dispersions and liquid crystalline phases, and thus can be used for any polymer/biopolymer. Variation of the spinning dope and the composition of the coagulation bath influence fiber properties, allowing the production of fibers with tunable mechanical properties. One disadvantage of wet spinning is the necessity to remove the solvent or coagulation bath residues after spinning, which requires at least one washing step resulting in a longer and therefore more expensive process compared to dry spinning (Jestin and Poulin 2014).

Besides 100 % of methanol or isopropanol (Adrianos et al. 2013; Albertson et al. 2014; An et al. 2011; Jones et al. 2015; Zhu et al. 2010), mixtures of water with monohydric alcohols are often used as coagulation baths (Arcidiacono et al. 2002; Bogush et al. 2009; Heidebrecht et al. 2015; Lazaris et al. 2002; Teule et al. 2007; Xia et al. 2010). The addition of water to the coagulation bath slows down the coagulation rate of spidroins, and water works as a plasticizer for the fibers, which renders them less brittle and prevents clogging of the spinneret (Lin et al. 2013).

Posttreatment, such as drawing the spun fibers in air or inside a bath, is applied to improve the fibers' mechanical properties. Poststretching of spun fibers has been shown to induce a higher β-sheet content (An et al. 2011) and to align the β-sheet crystals along the thread axis (Heidebrecht et al. 2015). In contrast to the coagulation bath, the poststretching bath needs to contain water because of its plasticizing features for the fibers, which enables the proteins to rearrange and align along the fiber axis. The absence of water results in brittle fibers. An overview of recombinant spider silk fiber wet spinning and posttreatment conditions is given in Table 1.

Electrospinning

Electrospinning of recombinant or reconstituted spider silk protein solutions is possible using an electric field of 4–30 kV with a distance of 2–25 cm between the electrodes (i.e., the capillary tip and the collector) (Bini et al. 2006; Bogush et al. 2009; Lang et al. 2013; Peng et al. 2009; Stephens et al. 2005; Wong Po Foo et al. 2006; Yu et al. 2014; Zhou et al. 2008; Zhu et al. 2015). Parameters influencing the fiber properties (e.g., fiber diameter) of nonwoven mats mostly depend on the properties of the spinning dope, such as the viscosity, surface free energy, protein concentration, and the solvent's intrinsic electrical conductivity and permeability (Greiner et al. 2006). In contrast to wet spinning, electrospinning of comparatively low protein concentrations of 2–6 % (*w*/*v*) (Bini et al. 2006; Leal-Egana et al. 2012; Wong Po Foo et al. 2006; Yu et al. 2014; Zarkoob et al. 2004) also yields fibers, but higher protein concentrations of 10–30 % (*w*/*v*) (Bogush et al. 2009; Lang et al. 2013; Leal-Egana et al. 2012; Peng et al. 2009; Stephens et al. 2005; Zhou et al. 2008; Zhu et al. 2015) are more commonly used. In general, increasing the spidroin concentration in the dope leads to an increased fiber diameter and a reduction of bead formation, the latter being an unwanted side effect of electrospinning (Lang et al. 2013; Leal-Egana et al. 2012). Structural analysis of nonwoven mats electrospun from HFIP using Fourier-transformed infrared spectroscopy (FTIR) with subsequent Fourier self-deconvolution (FSD) revealed a low β-sheet content (~15 %) (Lang et al. 2013). The electric field interacts with the hydrogen bond dipoles of the protein, stabilizing α-helical segments and thus inhibiting β-sheet formation (Stephens et al. 2005). Instead of a solid collector, water- or organic solvent-based coagulation baths can be used to collect the spun micro- and nanofibers. In general, the latter approach has the advantage of including a posttreatment within the spinning process. Yu et al. used a coagulation bath containing 90 % (*v*/*v*) of organic solutions (acetone or methyl alcohol) as a collector; however, SEM images showed inhomogeneous fibers containing many beads (Yu et al. 2014). Posttreatment of electrospun fibers with organic solvents or alcohols is necessary in order to render the spun α-helical fibers water insoluble (i.e., inducing β-sheet formation) (Lang et al. 2013; Leal-Egana et al. 2012; Slotta et al. 2006). Immersing the fibers into alcohol baths resulted in fused intersections of single fibers (Bini et al. 2006), giving the fibers a "molten" appearance. Therefore, instead of immersing the fibers, Leal-Egana et al. (2012) and Lang et al. (2013) exposed them to methanol or ethanol vapor to render the fibers water insoluble with keeping their original morphology.

Other spinning methods

Besides wet spinning and electrospinning, recombinant spider silk fibers were produced using microfluidic devices (Rammensee et al. 2008). Such devices mimic some aspects of the natural spinning process, such as ion exchange, pH change, and elongational flow conditions. Since only low or medium protein concentrations were used, high flow rates were necessary to induce fiber assembly. Shear forces can also be applied by hand-drawing fibers from pre-assembled spidroins out of aqueous solutions (Exler et al. 2007; Teule et al. 2007). The gained fibers show similar properties as those produced by wet spinning. However, several parameters can be fine-tuned within the microfluidic channels which will allow for more sophisticated spinning processes and, therefore, fibers, in the future.

Transgenic silkworms producing silkworm/spider silk composite fibers

One elegant way to "artificially" spin spider silk fibers is to use transgenic, naturally fiber-producing animals. Silkworms are naturally able to produce and spin silk proteins and they can be genetically modified. Transgenic silkworms were engineered to produce silkworm fibroin/spider silk composite fibers with a spider silk content of 0.4 to 5 % (*w*/*w*) (Kuwana et al. 2014; Teule et al. 2012). Importantly, the mechanical properties of silkworm silk (toughness 70 MJ m^{-3}; Gosline et al. 1999) are inferior to those of spider silk (toughness 167 MJ m^{-3}; Heidebrecht et al. 2015), and since the composite material merges the properties of both silks, the mechanical properties of hybrid silkworm/spider silk fibers will always be inferior to those of spider silk. In 2000, Tamura et al. succeeded in a stable germline transformation of the silkworm

Table 1 Overview of wet-spinning conditions used for generating recombinant spider silk fibers

Spinning dope	Max. protein concentration [%]	Coagulation bath	Posttreatment	Source
Aqueous				
160 mM or 1 M urea, 10 mM NaH_2PO_4, 1 mM Tris, 20 mM NaCl, 10 mM or 100 mM glycine, pH 5.0	25 (after ultrafiltration)	MeOH/H_2O mixture	N/A	Arcidiacono et al. (2002)
60 % NaNCS, 20 % acetate solution, mix ratio 8:2 or 10 % LiCl in 90 % formic acid (FA)	30	96 % EtOH	1st draw: 92 % EtOH 2nd draw: 75 % EtOH	Bogush et al. (2009)
50 mM Tris/HCl, pH 8.0 or 50 mM Na-phosphate buffer, pH 7.2	17	IPA/H_2O mixture	IPA/H_2O mixture	Heidebrecht et al. (2015)
0.1 % propionic acid, 10 mM imidazole; microwaved	12	100 % IPA	1st draw: 80 % IPA 2nd draw: 20 % IPA	Jones et al. (2015)
PBS	28	MeOH/H_2O mixture	1st draw: MeOH 2nd draw: H_2O	Lazaris et al. (2002)
Organic				
HFIP (5 % *v/v* added to dope prior to spinning)	15	100 % IPA	80 % IPA	Adrianos et al. (2013)
HFIP (evaporation of HFIP prior to spinning)	60	100 % IPA	85 % IPA, 60 °C	Albertson et al. (2014)
HFIP	30	100 % IPA	75 % IPA	An et al. (2011)
HFIP	12	IPA	N/A	Brooks et al. (2008)
HFIP	10	100 mM $ZnCl_2$, 1 mM $FeCl_3$ in H_2O	1st draw: air 2nd draw: 50–70 % EtOH	Lin et al. (2013)
HFIP (addition of 15 % water prior to spinning)	30	90 % IPA	N/A	Teule et al. (2007)
HFIP	15 (10 % silkworm fibroin and 5 % spider silk-like protein)	MeOH	1st: 3 h incubation in MeOH 2nd: drawing in distilled H_2O	Zhu et al. (2010)
HFIP	20	90 % MeOH	90 % MeOH	Xia et al. (2010)

MeOH methanol, *EtOH* ethanol, *IPA* isopropyl alcohol, *PBS* phosphate buffered saline, *HFIP* 1,1,1,3,3,3-hexafluoro-2-propanol, *N/A* not applicable

Bombyx mori using a *piggyBac*-derived vector (Tamura et al. 2000). *PiggyBac* is a transposon discovered in the lepidopteran *Trichoplusia ni* (Cary et al. 1989), and vectors based hereon are able to transpose into *B. mori* chromosomes enabling silkworm transformation with various genes encoding fibrous proteins (Tamura et al. 2000). In general, to create transgenic silkworms producing chimeric silkworm/spider silk, genes were designed encoding synthetic spider silk-like sequences, *B. mori* fibroin sequences as well as a *B. mori* promoter, targeting the foreign protein production to the silk gland. Subsequently, these genes were cloned into a *piggyBac*-based vector which was then injected into *B. mori* eggs. Silk fibroin fibers are composed of three proteins, namely fibroin heavy chain (H-chain), fibroin light chain (L-chain), and fibrohexamerin protein (fhx/P25) (Kojima et al. 2007), and they are covered by a sericin layer (Wen et al. 2010). Just like spidroins, silk fibroins consist of a highly repetitive region which is flanked by nonrepetitive amino- and carboxy-terminal domains. Since the H-chain is believed to be mainly responsible for the mechanical properties of the silk (Kojima et al. 2007), fibroin H-chain genes were modified with spider silk sequences for improved properties (Kuwana et al. 2014; Teule et al. 2012; Zhu et al. 2010). Kuwana et al. (2014) generated three transgenic silkworm strains using a Japanese commercial silkworm strain (C515), two of which contained cDNA of major ampullate spidroins of the spider *Araneus ventricosus*, flanked by the amino- and carboxy-terminal domains of the *B. mori* fibroin H-chain gene. The third strain consisted of a plasmid coding for enhanced green fluorescent protein (EGFP), in order to simplify the analysis of the spun cocoons, subcloned in between the amino- and carboxy-terminal domains of the H-chain gene. After creating transgenic silkworms carrying the modified genes using the *piggyBac*-based vector system, the silkworms produced the modified H-chain/spider silk protein in the silk gland. In the silkworm's gland, the modified H-chain protein dimerized with the fibroin L-chain and was subsequently spun into a cocoon containing the spider dragline protein (Kuwana et al.

2014). Transgenic efficiencies of the strains were 20.0 % for the EGFP-containing strain and 16.7 and 22.6 %, respectively, for the strains containing the spider silk cDNA. Cocoons of the EGFP-transgenic silkworms showed green fluorescence, indicating that the EGFP protein is folded in its functional structure after spinning, suggesting that the spider silk protein may also be present in the cocoon fibers in its natural structure. The maximum amount of the modified H-chain/spider silk protein against the total fibroin was estimated at 0.4–0.6 % (*w*/*w*) (Kuwana et al. 2014) and 2-5 % (*w*/*w*) (Teule et al. 2012).

Alternatively, the sericin promoter has been used to target spider dragline silk proteins toward the outer sericin layer of the silk fiber (Wen et al. 2010). Whereas the breaking strain of the composite fiber was similar to that of natural spider silk fibers, the breaking stress and toughness were increased compared to that of natural silkworm silk, but the average values were still well below those of natural spider silk fibers (Teule et al. 2012). Theoretically, a breaking stress of cocoon silk equal to that of spider dragline silk could be achieved if the spidroin content was raised to 5–8 % (Kuwana et al. 2014), but this has not been shown experimentally, yet.

Properties of reconstituted vs. recombinant fibers

In order to establish processing technologies for gaining biomimetic spider silk fibers, two research groups used reconstituted *Nephila* spp. dragline silk for fiber spinning (Seidel et al. 2000; Shao et al. 2003). The best performing fibers, in terms of mechanical properties, were obtained by drawing the fibers in air after spinning, soaking them in water, and then drawing them in air again. These fibers exhibited a strength of 320 MPa, a Young's modulus of 8 GPa, and an extensibility of 100 % (Seidel et al. 2000). In comparison to natural dragline fibers, these fibers were much more extensible, but had a lower strength. Hand-drawn fibers of reconstituted *Nephila edulis* dragline silk yielded fibers showed natural dragline-like extensibility (10–27 %) and Young's modulus (6 GPa), but a breaking strength (100–140 MPa) that was well below that of the natural dragline fibers (Shao et al. 2003). Generally, achieving man-made fibers with a breaking stress in the regime of natural spider silk fibers seems to be the greatest challenge. Since the amount of natural and, therefore, also reconstituted, spider silk is quite limited due to the facts as mentioned above, the generation of recombinant (i.e., artificial) silk fibers is the only meaningful route toward large-scale applications. In several attempts, extensibility (1.2–302 %) and Young's modulus (0.04–21 GPa) of artificial spider silk fibers have been highly variable, reaching lower as well as higher values in comparison to natural spider silk fibers (24 % and 8 GPa). On the other hand, the strength even of the best performing fibers achieved values far below those of natural spider silk fibers. The highest strength (660 MPa) was achieved by silkworm/spider silk composite fibers, but since these fibers were only extensible up to 19 % (Wen et al. 2010), the toughness was far below that of natural spider silk fibers. In comparison, the highest strength (508 MPa) of recombinant spider silk fibers was achieved by wet spinning of proteins with a molecular weight of 285 kDa containing only amino acid motifs based on the core domain of natural spidroins (Xia et al. 2010). The highest toughness (189 MJ m^{-3}), on the other hand, was observed with fibers wet-spun from a self-assembled aqueous spinning dope of a 134-kDa protein containing all three functional domains: the highly repetitive core domain as well as the helical amino- and carboxy-terminal domains (Heidebrecht et al. 2015).

Tensile testing of electrospun, recombinant fibers also showed, not surprisingly, inferior mechanical properties in comparison to those of natural spider silk fibers (Bogush et al. 2009; Zhu et al. 2015). But in this case, mechanics can be neglected, since electrospun fibers are commonly applied as nonwoven meshes used for biomedical or for filter applications without the need of nature-like mechanical properties. In this context, biocompatibility is the more important feature of spider silks. In general, fibers produced from both reconstituted and recombinant spidroins exhibited good biomedical properties. For instance, fibers electrospun from reconstituted *A. ventricosus* major ampullate spidroins revealed a very low degradation rate and showed a good biocompatibility with PC 12 cells (Yu et al. 2014). Cell attachment and proliferation experiments of BALB/3T3 mouse fibroblasts on nonwoven meshes spun from recombinant spidroins showed cell alignment along individual fibers as well as a protrusion of filopodia/lamellipodia through the interstitial space between the fibers (Leal-Egana et al. 2012). Electrospinning of recombinant spidroins hybridized with the cell binding sequence RGD even induced the differentiation of bone marrow-derived, human mesenchymal stem cells (hMSCs) to osteogenic outcomes (Bini et al. 2006). Also, self-assembled recombinant spidroin fibers implanted subcutaneously in rats did not show any negative systemic or local reactions (Fredriksson et al. 2009), suggesting these fibers to be biocompatible. Additionally, fiber bundles thereof seem to support the formation of vascularized tissue formation, since already 1 week after implantation, new capillaries and fibroblast-like cells formed in the center of such fiber bundles (Fredriksson et al. 2009).

"Not to spin": artificial assembly morphologies

Recombinant spider silk proteins can be processed into more than fibers; other morphologies such as particles, foams, films, or hydrogels can also be fabricated, all of which have a high

application potential (Hardy and Scheibel 2010; Hermanson et al. 2007; Slotta et al. 2008; Spiess et al. 2010a, b). Processing of recombinant spider silk proteins in aqueous solutions can be triggered by changes in the pH value, amount and type of additives (e.g., potassium phosphate, alcohols, or polymers), mechanical shear, or temperature changes. Alternatively, organic solvents such as HFIP or FA can be used; however, the choice of solvent has a significant impact on structure formation. While aqueous processing leads mainly to particle and hydrogel formation, water-soluble films are mainly produced using fast-evaporating organic solvents. Here, posttreatment procedures with agents, like potassium phosphate or monohydric alcohols (methanol, ethanol, isopropanol), are necessary to render the films insoluble in water (Exler et al. 2007; Huemmerich et al. 2004a, b; Lammel et al. 2008; Numata et al. 2010; Rammensee et al. 2008; Rising 2014; Scheibel 2004; Slotta et al. 2007; Spiess et al. 2010b).

Particles

Spidroin particles are produced in a simple, aqueous process by the addition of high concentrations of kosmotropic salts, like potassium phosphate, and fast mixing. This procedure results in solid particles with high β-sheet content, a smooth surface (Hofer et al. 2012; Lammel et al. 2008; Slotta et al. 2008), and particle sizes between 250 nm and 3 μm depending on the mixing intensity, protein concentration, and the concentration of kosmotropic salts (Lammel et al. 2008; Slotta et al. 2008; Spiess et al. 2010a). Using ionic liquids instead of aqueous buffers and high potassium phosphate concentrations to induce phase separation and nucleation in the protein solution or ultrasonication for particle production allowed enhanced size control and a reduced polydispersity index (Elsner et al. 2015; Lucke et al. 2015). eADF4(C16) (engineered *Araneus diadematus* fibroin 4) particles show a brush-like outer layer with protruding protein strands and a thickness of 30–50 nm covering a solid inner core (Helfricht et al. 2013). Importantly, no posttreatment with dehydrating agents is necessary to obtain water-insoluble particles, since the β-sheet content is high after the salting-out process (Slotta et al. 2008). Further, it has been shown that particles made of recombinant spider silk proteins exhibit an extraordinary mechanical stability when analyzed in dry state. In a swollen, hydrated state, these particles exhibited a different mechanical behavior: the elastic modulus was three orders of magnitude lower (E modulus dry, 0.8±0.5 GPa; E modulus hydrated, 2.99±0.90 MPa). Further, when dry, the particles deformed in a plastic response, and when hydrated, they showed a reversible, elastic deformation behavior. In both states, dry and hydrated, the mechanical properties were dependent on the molecular weight of the spidroin: The higher the molecular weight, the better the mechanical stability (Neubauer et al. 2013).

Particles made of recombinant spider silk proteins are suitable for a large variety of applications. Due to their enhanced mechanical properties, these particles can be used, for example, as filler for composite materials. Additionally, due to their favorable properties in a physiological environment (nontoxic, biodegradable, etc.), these particles could be used as carriers of different substances, for example in drug delivery. Silk particles retain their properties for a limited period of time in the human body before they gradually decompose into degradation products which can be eliminated (Altman et al. 2003; George and Abraham 2006; Liu et al. 2005; Müller-Herrmann and Scheibel 2015).

Uptake and release studies of small molecules with model drugs showed that these types of molecular entities can be incorporated either by diffusion or by coprecipitation of both the spidroin and the drug substance. While the latter increased the loading efficiency of the particles, it did not significantly influence the release rate. Importantly, drugs can be only loaded into spidroin particles if there is no electrostatic repulsion. In this context, only positively and neutrally charged drugs can be loaded onto negatively charged spider silk protein particles, such as those made of eADF4(C16) (Blüm and Scheibel 2012; Doblhofer and Scheibel 2015; Lammel et al. 2011). Since protein design allowed the production of positively charged spider silk proteins, particles made thereof were also able to uptake negatively charged small molecules as well as large oligonucleotides (Doblhofer and Scheibel 2015).

One important justification for the use of silk-based drug delivery vehicles is the ability to design the underlying proteins for a specific target, for example uptake by a specific cell type. Previous investigations showed that, in general, negatively charged spider silk particles have a low uptake efficiency by mammalian cells. Therefore, cell penetrating peptides (CPP) as well as an Arg8-TAG or a RGD sequence were engineered to the N- and C-termini of eADF4(C16). The presence of CPP increased the number of incorporated particles in HeLa cells; however, the mechanism behind the increased uptake was surprisingly mainly the particle's surface charge, not the presented surface peptide (Elsner et al. 2015).

Films

The first studies on films made of spider silk proteins were reported in 2002 by Chen et al. where the salt-controlled structural conversion of natural spider silk proteins obtained from the major ampullate gland of *Nephila senegalensis* was investigated (Chen et al. 2002). Films made of recombinant spider silk proteins first gained attention in 2005 where it was shown that these spider silk-like proteins undergo a similar structural conversion from random coil to β-sheet rich. Recombinantly produced engineered spider silk protein films turned out to be

transparent and chemically stable under ambient conditions, depending on their processing (Huemmerich et al. 2006; Slotta et al. 2006; Spiess et al. 2010b). Two major components determine the properties of these films: the molecular structure including the secondary structure and intermolecular as well as intramolecular interactions as well as the macroscopic structure reflecting the material's interface with the environment (Spiess et al. 2010a).

Depending on the solvent, spider silk proteins in solution adopt mainly an α-helical or random coil conformation which is often maintained in as-cast films (Borkner et al. 2014; Slotta et al. 2006). These as-cast films are water soluble, as mentioned above, due to the weak intermolecular interactions of spider silk proteins in an α-helical conformation. Upon conversion of the protein structure toward a β-sheet-rich structure by using agents like kosmotropic salts or alcohols, water vapor, or temperature annealing, films can be rendered chemically more stable and thereby water insoluble (Huemmerich et al. 2006; Slotta et al. 2006; Spiess et al. 2010b). This is an important quality, as most potential applications of recombinant spider silk films involve interaction with a humid environment. Structural control over synthetic recombinant spider silk proteins is also given by the variation of the amino acid sequence toward a higher number of β-sheet forming building blocks, and with the control of the β-sheet portion, mechanical properties can be predetermined (Rabotyagova et al. 2009, 2010). While the terminal domains of spider silk proteins play an important role in the fiber spinning process, no significant influence of the nonrepetitive regions could be observed during the film casting from organic protein solutions. Nevertheless, as-cast films made of recombinant spider silk proteins containing the NR regions are slightly more chemically stable than those without, though there are no disulfide bridges present (Slotta et al. 2006). Besides chemical stability, the β-sheet content also determines the mechanical properties of a film. With increasing β-sheet content, elastic modulus and strength increase, and there is a loss of elasticity. High amounts of β-sheets, therefore, can be correlated with stiffness and brittleness in silk films (Spiess et al. 2010b). However, as the content of β-sheets can be adjusted by the posttreatment conditions upon varying incubation times of the films in alcohols or posttreatment with water/alcohol mixtures at various ratios lead to a varying β-sheet content, this is not a challenge for tailoring films to specific applications (Spiess et al. 2010a). The water content in silk films plays also an important role; due to its softening effect, it can work as a plasticizer. Another possibility to overcome the brittleness of silk films is to add plasticizers like glycerol. It was reported that glycerol can alter the intermolecular interactions of silk proteins in a film and, therefore, is able to enhance the films' elasticity. The addition of 40 % *w*/*w* glycerol to an eADF4(C16) film yielded a 10-fold increased elasticity, but also going along with a 10-fold decrease of the elastic modulus and a slight decrease in strength (Lawrence et al. 2010; Spiess et al. 2010a).

Spider silk protein films can be envisioned for various applications; however, they are especially promising for use in the biomedical field due to their biocompatibility which has been demonstrated in vitro and in vivo (Allmeling et al. 2006, 2008; Gellynck et al. 2008a, b; Hakimi et al. 2010; Vollrath et al. 2002). Conceivable applications are materials for a controlled substance release at a specific site of action in the human body, biomedical sensors, and cell-supporting scaffolds (Hardy et al. 2013; Minoura et al. 1995; Sofia et al. 2001; Vendrely and Scheibel 2007). It is possible, for example, to directly integrate substances (e.g., drugs) into silk films or to load these substances into microparticles that are then embedded in or coated with a silk layer amenable for delayed release (Wang et al. 2007, 2010). Biomedical or biochemical sensors can be fabricated by covalent binding of biologically active compounds to the silk proteins (Lawrence et al. 2008; Spiess et al. 2010b). Cell adhesion on recombinant spider silk protein scaffolds was shown to be very weak (Baoyong et al. 2010); therefore, chemical or genetic coupling of specific functional groups, for example components of the extracellular matrix, and modification of the surface hydrophilicity have been employed to influence the cellular response to a film's surface concerning adhesion, proliferation, and differentiation (Bini et al. 2006; Karageorgiou et al. 2004; Wohlrab et al. 2012a). As mentioned above, the function of silk films can be also partly controlled by the macroscopic structure they adopt. Changing the surface morphology by patterning or partial roughening of a film under different posttreatment conditions can lead to a deviating behavior of cells thereon (Bauer et al. 2013; Borkner et al. 2014). The hydrophilicity of the film surface can easily be affected by the choice of the template for drop-cast films (Wohlrab et al. 2012b). The influence of the template's surface hydrophilicity can be diminished by spin coating of spidroin solutions, since the duration of solvent evaporation determines the rearrangement of silk protein molecules within the films, and their interaction with the underlying substrate and the film properties are in this case determined by the utilized solvent (Metwalli et al. 2007; Wohlrab et al. 2012b). Applications of films made of silk protein in the medical field include coatings for medical devices (Bettinger and Bao 2010; Kim et al. 2010; Zeplin et al. 2014a, b) and skin grafts (Baoyong et al. 2010; Jiang et al. 2007).

In the context of biomedical applications, it is important to mention that recombinant spider silk protein films undergo partial degradation in the presence of wound proteases (~10 %) in a timescale of 15 days, which is in the range of

the initial phase of wound healing (Müller-Herrmann and Scheibel 2015).

Hydrogels

Hydrogels are three-dimensional polymer networks that absorb over 95 % (*w*/*w*) of water (Knight et al. 1998; Lee and Mooney 2001; Rammensee et al. 2006; Schacht and Scheibel 2011; Shin et al. 2003). Their porous structure and mechanical properties make them candidates for applications in tissue engineering, drug delivery, or as functional coatings (Rammensee et al. 2006). The mechanical properties of a specific hydrogel are determined by the properties of its individual constituents, and many different polymers, synthetic and natural ones, have been utilized to form hydrogels. Spidroin hydrogels are built upon self-assembly of nanofibrils by a mechanism of nucleation-aggregation followed by a concentration-dependent gelation in which β-sheet-rich spider silk fibrils become entangled to build a stable three-dimensional network (Hu et al. 2010; Rammensee et al. 2006; Schacht et al. 2015; Schacht and Scheibel 2011; Slotta et al. 2008). Spider silk proteins can be processed into stable hydrogels in a controlled manner by adjusting the protein concentration, pH, temperature, ion composition, and concentration (Jones et al. 2015; Schacht and Scheibel 2011; Vepari and Kaplan 2007). Each of these inputs influences the hydrogel's morphology, mechanical properties, and pore size. In particular, increasing the protein concentration and increasing the addition of chemical crosslinkers lead to an increase in mechanical strength, accompanied by a decrease in pore sizes (Schacht and Scheibel 2011). It has been recently shown that recombinant spidroin hydrogels, like many biopolymer hydrogels, show a viscoelastic behavior with stress changes proportional to the linear increasing strain (Mackintosh et al. 1995). In the special case of eADF4 hydrogels, the elastic behavior dominates over the viscous behavior, with low-viscosity flow behavior, good form stability, and a shear thinning effect, allowing their use as bioink in a biofabrication setup. Eukaryotic cells were embedded within the hydrogel prior to printing with a bioplotter and they survived for at least 7 days after printing. The addition of cells did not considerably influence the print-ability of the spider silk protein gels (Schacht et al. 2015).

Foams and sponges

Foams are defined as material containing small bubbles formed on or in a liquid. To produce foams made of spidroin solutions, gas bubbles remain stable when using a high protein concentration, and the foam is established upon drying. In comparison, sponges are, like foams, three-dimensional porous scaffolds, but differ in their production technique and their mechanical properties. Sponges can be produced by gas foaming, lyophilization, or using porogens. It has been shown for silkworm silk fibroin that porogens like sodium chloride and sugar can be used to produce sponges with defined pore sizes due to silk β-sheet formation around the porogen. Therefore, the pores are the size of the porogen in case of organic protein solutions and 80–90 % of the size of the porogen in aqueous solutions (Kim et al. 2005). As a consequence, it is even possible to produce pore size gradients by stacking porogens with different diameters (Kim et al. 2005; Nazarov et al. 2004; Vepari and Kaplan 2007). Foams and sponges are both qualified for cell culture applications due to the ability of good transportation of nutrients and metabolic waste through the material in combination with a good structural and mechanical stability (Kluge et al. 2008). While a number of studies on silkworm silk fibroin foams and sponges have been published, spider silk protein foams and sponges remain largely unexplored. Widhe et al. showed in 2010 that their recombinant miniature spider silk protein 4RepCT can be processed into foams which stay microscopically stable in a cell culture medium. The surface of these foams showed heterogeneous pores with diameters between 30 and 200 μm. However, in this pore size range, foams lack a characteristic surface topography which influences cell adhesion (Widhe et al. 2010).

Concerning spider silk sponges, Jones et al. developed a method in which hydrogels were frozen in an aqueous medium and subsequently thawed, resulting in a highly elastic, three-dimensional morphology. Such sponges could uptake water to the extent of hydrogels as well as maintain their form upon compression and drying. That is, the effect of dehydration was completely reversible by the addition of water. The high elasticity of these sponges is based on a lower content of stiffening β-sheet crystals and a higher amount of the elastic random coil and helical structures in comparison to other spider silk scaffolds (Jones et al. 2015).

Composite materials including spider silk

Composites provide the opportunity to produce materials with extraordinary properties by complementation of at least two different kinds of materials. In this context, natural as well as recombinant spider silk materials can play a role due to their outstanding mechanical and biocompatible properties. In some studies, naturally spun spider dragline silks were used to assemble composites with inorganic nanoparticles to reinforce the fibers. Recently, it was shown that feeding spiders with carbon nanotubes or graphene dispersions led to carbon-reinforced silk threads (Lepore et al. 2015). Despite that, most approaches to enhance mechanical strength of spider dragline silk were employed after collection of the silk by forced silking. Dragline silk was used as template for the insertion of zinc (Zn), titanium (Ti), and aluminum (Al) by multiple pulsed vapor-phase infiltration (MPI). This treatment

increased the toughness of the spider silk fibers by almost 10-fold and the E moduli of the fibers from 9.7 to 68 GPa, in the best case (Lee et al. 2009).

Spider silk composite production allows not only to increase its mechanical strength but also to extend the range of applications. Dragline silk fibers, for instance, were incubated in chloroauric acid to assemble gold nanoparticles on their surface with the goal of producing water and methanol vapor sensors with a response time of about 10 s and 150-fold change of conductivity. Their supercontraction behavior in the presence of water and methanol vapor led to a change in the distance of the gold nanoparticles and, therefore, altered the electrical conductivity of the fibers (Hardy and Scheibel 2010; Singh et al. 2007). Electrical conductivity was also introduced into spider silk fibers by deposition of amine-functionalized multiwalled carbon nanotubes (MWCNT) onto their surface. In this study, additionally, an increase in mechanical strength was observed for the composite fibers. The combination of properties allowed an extended application of the material in various technical approaches (Steven et al. 2013). The accumulation of calcium carbonate or hydroxyapatite (HAP) on naturally spun fibers enabled producing new scaffolds for bone tissue engineering or building blocks for bone replacement materials (Cao and Mao 2007; Mehta and Hede 2005). In the case of hydroxyapatite deposition, oriented crystal growth was obtained being consistent with the orientation of β-sheet crystals in the silk fibers (Cao and Mao 2007). In another approach, naturally spun spider silk was solubilized in FA for electrospinning. By mixing the resulting protein solution with a poly(D,L-lactic acid) (PDLLA) FA solution and subsequent electrospinning, nonwoven meshes with core-shell structured fibers with a diameter range of about 90–1000 nm were produced. The size of the fibers was tuned by the weight ratio of the two material components in the spinning solution (Zhou et al. 2008).

Recombinant spider silk proteins have been used in blends with polycaprolactone (PCL) and thermoplastic polyurethane (TPU) to cast films with a higher elasticity than nonblended spider silk protein films. Good cell adhesion, proliferation, and the possibility to incorporate drugs in these composite films endorse them as candidates for implant coatings or as scaffolds for tissue engineering (Hardy et al. 2013). Another filler material used in spider silk protein films were carbon nanotubes. Composite films made of recombinant spider silk proteins and single-walled carbon nanotubes led to excellent mechanical properties as a result of the transfer of stress in the matrix to the filler and of the potential for extensive reorganization of the matrix at applied high stress (Blond et al. 2007).

Blended dopes of recombinant spider silk with collagen or gelatin have also been used for electrospinning processes. The resulting composite nonwoven meshes were predominantly used in tissue engineering. Electrospinning of a mixture of spider silk proteins and collagen led to unidirectional, partially crosslinked fiber scaffolds usable in stem cell differentiation and in neural tissue engineering. Collagen-dominant scaffolds were found to provide unique structural, mechanical, and biochemical cues; stem cells were directed to neural differentiation, and the development of long neural filaments along the fibers was facilitated. These neural tissue-like constructs are promising candidates for transplantation in cellular replacement therapies for neurodegenerative disorders such as Alzheimer's or Parkinson's disease (Sridharan et al. 2013; Zhu et al. 2015). Tubular scaffolds made of a blend of recombinant spider silk proteins and gelatins, supported by a polyurethane outer layer, were produced to be used in tissue-engineered vessel grafts (TEVG). The morphological and mechanical characterization of the tubular structures showed strong similarities with the structure of native arteries, both in strength and elasticity. The appearance of RGD sequences in spider silk used for this purpose supported the growth of adult stem cells, yielding a higher cellular content prior to prospective implantation than without the cellular recognition sequence (Zhang et al. 2014).

Modification of recombinant spider silk proteins with specific binding motifs for HAP (Huang et al. 2007), titanium dioxide, germania, and gold could be assembled into various morphologies and provided the control of organic-inorganic interfaces and composite structural features (Belton et al. 2012; Foo et al. 2006; Mieszawska et al. 2010). Silica binding sequences (e.g., R5 from *Cylindrotheka fusiformis*) were used to control silica particle formation and assembly on the surfaces of spider silk films, fibers, and particles. Mineral phase formation, morphology, chemistry, and, therefore, composite properties could be influenced by varying the processing conditions or by sequence alteration. Silica is a critical osteoconductive element, which can be processed under ambient conditions, and has the potential to control the tissue remodeling rate, making this composite a possible scaffold for bone regeneration. Studies with human mesenchymal stem cells (hMSCs) attached to silica/silk films showed upregulation of osteogenic gene markers at high silica contents (Belton et al. 2012; Foo et al. 2006; Mieszawska et al. 2010).

Summary and outlook

Biotechnological production of spider silk proteins and their processing into diverse morphologies (Fig. 4) allow for applications in textile, automotive, and biomedical industries. Concerning the production of artificial spider silk fibers, significant progress has been made in the last years. Since reconstituted spider silk fibers did not show nature-like mechanical properties after spinning, various techniques for biotechnological production (i.e., proteins, transgenic animals, etc.) have been investigated to gain proteins enabling fibers with such features. Regarding the biotechnological production

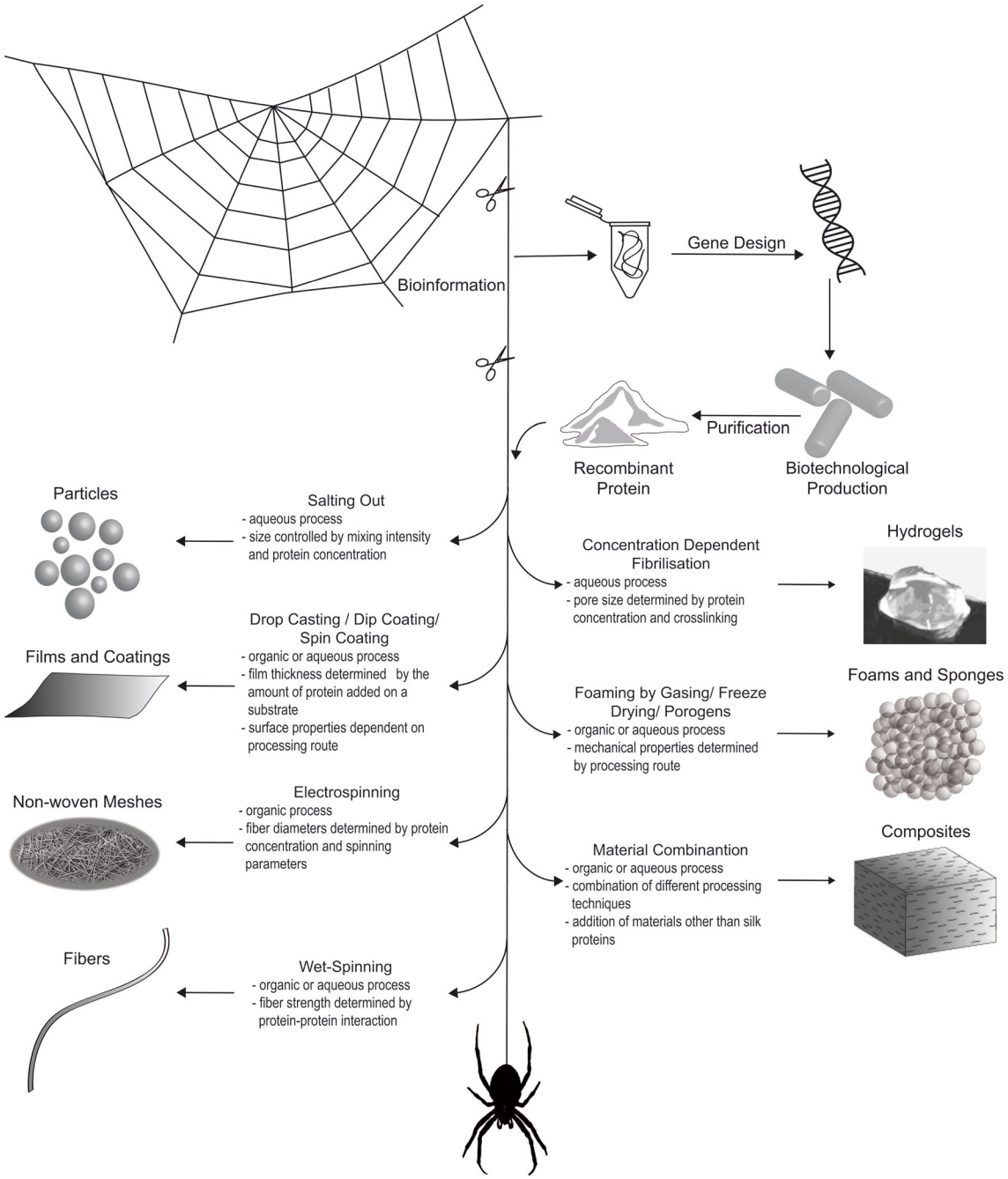

Fig. 4 Design, production, and processing of recombinant spider silk proteins: from identification of the bioinformation given by the natural material produced by a spider, to genetic design of its recombinant counterpart, to possible morphologies

and artificial fiber spinning, great progress was made by analyzing the natural spinning process and the role of the amino- and carboxy-terminal domains. Inclusion of the nonrepetitive terminal domains into the recombinantly produced spider silk proteins and wet spinning these proteins into fibers resulted in a toughness comparable to that of natural fibers. This emphasized the importance of the nonrepetitive terminal domains in the proper alignment of the spidroins, which was neglected in earlier trials. By fine-tuning the composition of the recombinant proteins and the spinning process, artificial spider silk fibers with mechanical properties exceeding those of the natural fibers will be likely possible in the future.

Recombinant production of spider silk proteins does not only offer the option to mimic nature and produce fibers that are similar to their natural counterparts, but it also enables the production of different morphologies. These different structures are biodegradable and biocompatible just like the natural equivalents, but still comprise new properties that lead to

applications in both the medical and the technical field. Particles and films/coatings have already been well-investigated, and this paves the way toward the first applications in drug delivery and cell culture. On the other hand, hydrogels, foams, and sponges require further exploration before they can be used directly in applications. Nevertheless, in all cases, recombinant spider silk protein research tends to explore new tailor-made materials by adapting the morphology's properties to a specific application. The potential of recombinant spider silk proteins in different fields is thereby essentially limitless.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethics approval This article does not contain any studies with human participants or animals performed by any of the authors.

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