Chapter 10
The Power of Recombinant Spider Silk Proteins

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Abstract Due to their outstanding mechanical properties, their biocompatibility and biodegradability spider silk fibers are of high interest for researchers. Silk fibers mainly comprise proteins, and in the past decades biotechnological methods have been developed to produce spider silk proteins recombinantly in varying hosts, which will be summarized in this review. Further, several processing techniques like biomimetic spinning, wet-spinning or electro-spinning applied to produce fibers and non-woven meshes will be highlighted. Finally, an overview on recent developments concerning genetic engineering and chemical modification of recombinant silk proteins will be given, outlining the potential provided by recombinant spider silk-chimeric proteins and spider silk-inspired polymers (combining synthetic polymers and spider silk peptides).

Keywords Recombinant spider silk proteins • Fibers • Genetic engineering • Chemical modification • Biopolymer

10.1 Introduction

Female orb weaving spiders such as *Nephila clavipes* and *Araneus diadematus* are able to produce up to seven different silk types for different applications, such as catching and wrapping prey, for protecting their offspring etc. Spider silks are mainly made of silk proteins (spidroins) and they are named after the glands the proteins are produced in. The best characterized silk is the Major Ampullate (MA)

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silk (also known as dragline silk). In order to analyze the properties of spider silk as well as to use them in distinct applications, sufficient quantities of the material are necessary.

Unfortunately, due to their cannibalistic behavior it is not possible to farm spiders. Further, collecting silk from individual spiders is time consuming and not very effective. Therefore, silk genes have been transferred from spiders to other host organisms to produce recombinant spider silk proteins. Besides employing cDNA, engineered silk genes have been utilized encoding proteins which are comparable to the natural ones concerning their amino acid sequence.

Here, we summarize efforts to recombinantly produce spider silk proteins in bacteria and highlight the possibilities of their application-driven modification, as well as the techniques of processing them into fibers.

10.2 Recombinant Production of Dragline Silk Proteins

Dragline silk comprises spidroins produced in the major ampullate gland and is used e.g. as the frame and the radii of an orb web, and also as the spider's lifeline. In comparison to most man-made fibers like Kevlar or carbon fibers, dragline silk has superior mechanical properties especially concerning its toughness (Heim et al. 2009; Gosline et al. 1999). Two major ampullate spidroin classes can be defined named MaSp 1 and MaSp 2, which mainly differ in proline content (MaSp1 shows no or little proline residues, while MaSp2 is enriched in proline residues). Furthermore, MaSp1 tends to be more hydrophobic than MaSp2. The molecular setup of MaSp proteins is given in Fig. 10.1 showing exemplary the MaSp2 protein ADF4 of *Araneus diadematus*.

Major ampullate spidroin monomers have a molecular weight of up to 350 kDa (Ayoub et al. 2007; Sponner et al. 2004). Despite of their different functions,

Fig. 10.1 Molecular setup of the recombinant protein eADF4 (C16) (engineered ADF4). A consensus repeat motif (Module C) is derived from the repetitive core of the dragline protein ADF4. By reverse translation, synthetic oligonucleotides can be produced and seamlessly cloned

spidroins comprise a highly repetitive core sequence, flanked by non-repetitive termini (Scheibel 2004; Eisoldt et al. 2011). The core domain accounts for approximately 90 % of the protein's sequence and consists of repeating motifs, each formed by 30–150 amino acids which can be repeated up to 100 times in a single spidroin (Hayashi et al. 2004; Guerette et al. 1996).

While the repetitive core domain of a spider silk protein is important for its macromolecular properties, the non-repetitive termini play a key-role in storage and assembly. Compared to the core domain, the carboxy (NRC)- and aminoterminal (NRN) domains are highly conserved for each silk type throughout different species or sometimes even between different silk types (Motriuk-Smith et al. 2005; Rising et al. 2006; Garb et al. 2010), indicating their highly important function. Both terminal domains of MaSp form five-helix bundles (Hagn et al. 2010, 2011; Askarieh et al. 2010). The recently studied NRN domains of *Latrodectus hesperus* and *Euprosthenops australis* are monomeric at pH above 6.8 and dimerize in an antiparallel fashion upon slight acidification. In comparison to the aminoterminal domains, the NRC domain of *Araneus diadematus* is a disulphide-linked, parallel dimer with one of the five helices being domain swapped. Importantly, all solution structures were solved using recombinantly produced proteins.

First attempts to produce recombinant spider silk proteins by using cDNA from spiders revealed several problems. Bacterial hosts such as *E. coli* have a different codon usage than spiders lowering the protein yield. Further repetitive sequences are often removed in bacteria by homologous recombination creating a polydisperse set of proteins with different molecular weight (Xu and Lewis 1990; Arcidiacono et al. 1998). Likewise, the efforts to express silk genes in eukaryotic cells were not successful or yielded low protein amounts (Menassa et al. 2004; Lazaris et al. 2002). A short overview on different host organisms used for recombinant spider silk protein production is shown in Table 10.1.

A more promising approach is the recombinant production of engineered spider silk spidroins with adapted DNA sequences. Based on the repetitive core sequence of spider silk spidroins, it is easy to design optimized genes by simple cloning techniques (Lawrence et al. 2004; Colgin and Lewis 1998; Huemmerich et al. 2004a; Lewis et al. 1996; Prince et al. 1995; Vendrely et al. 2008). An overview on recent attempts is shown in Table 10.2.

10.3 Processing of Recombinant Spider Silk Proteins

Spider silk proteins can be processed into fibers, capsules, particles, hydrogels, foams, films or non-woven meshes (Schacht and Scheibel 2011; Spiess et al. 2010; Leal-Egana et al. 2012; Hardy et al. 2008). Assembly of the recombinant spidroins can be triggered by protein concentration, pH, temperature, ionic strength or mechanical stress, among others.

Here we will focus on processing of recombinant spider silk proteins into fibers and non-woven meshes.

Table 10.1 Host organisms used for expressing spider cDNA and cDNA fragments

Host organism	DNA origin	MW [kDa] of the protein	Silk type	References
Prokaryotes				
E. coli	*N. clavipes*	43	MaSp1	Arcidiacono et al. (1998)
E. coli	*N. clavipes*	12	MaSp1 & MaSp2	Sponner et al. (2005)
E. coli	*N. antipodiana*	12–15	TuSp1	Lin et al. (2009)
E. coli	*E. australis*	10–28	MaSp1	Askarieh et al. (2010), Hedhammar et al. (2008), and Stark et al. (2007)
E. coli	*L. hesperus*	N/A	PySp2	Geurts et al. (2010)
E. coli	*L. hesperus*	33, 45	TuSp1	Gnesa et al. (2012)
Eukaryotes				
Insect cells (*S. fruiperda*)	*A. diadematus*	35–56	ADF3 & ADF4	Huemmerich et al. (2004b)
Insect cells (*S. fruiperda*)	*A. diadematus*	50–105	ADF3 & ADF4	Ittah et al. (2006)
Insect cells (*S. fruiperda*)	*A. ventricosus*	28	Flag	Lee et al. (2007)
Insect cells (*S. fruiperda*)	*A. ventricosus*	61	Polyhedron-Flag fusion protein	Lee et al. (2007)
Mammalian cells (MAC-T & BHK)	*N. clavipes*	12	MaSp1 & MaSp2	Lazaris et al. (2002)
Mammalian cells (MAC-T & BHK)	*A. diadematus*	60–140	ADF3	Lazaris et al. (2002)
Mammalian cells (COS-1)	*Euprosthenops* sp.	22, 25	MaSp1	Grip et al. (2006)
Transgenic animals (goats)	*A. diadematus*	60	ADF3	Karatzas et al. (1999)
Transgenic animals (mice)	*N. clavipes*	31–66	MaSp1 & MaSp2	Xu et al. (2007)
Transgenic animals (*B. mori*)	*N. clavipes*	83	MaSp1	Wen et al. (2010)
Yeast (*P. pastoris*)	*N. clavipes*	33–39	MaSp1 & MaSp2	Teulé et al. (2003)
Yeast (*P. pastoris*)	*N. clavipes*	57–61	ADF3	Teulé et al. (2003)

MaSp major ampullate spidroin, *ADF Araneus diadematus* fibroin, *MAC-T* bovine mammary alveolar cells, *BHK* baby hamster kidney cells, *COS* fibroblast-like cell line derived from monkey kidney tissue, *Flag* flagelliform, *TuSp* tubiliform spidroin, *PySp* pyriform spidroin, *MW* molecular weight

Table 10.2 Engineered spider silk proteins produced in different bacterial hosts

Silk type	Origin	MW [kDa] of the protein	Host organism	References
MaSp1	*L. hesperus*	N/A	*S. typhimurium*	Widmaier et al. (2009) and Widmaier and Voigt (2010)
	N. clavipes	100–285	*E. coli*	Xia et al. (2010)
	N. clavipes	15–26	*E. coli*	Winkler et al. (1999) and Szela et al. (2000)
	N. clavipes	45–60	*E. coli*	Huang et al. (2007), Wong Po Foo et al. (2006), and Bini et al. (2006)
	N. clavipes	10–20	*E. coli*	Fukushima (1998)
MaSp2	*A. aurantia*	63–71	*E. coli*	Brooks et al. (2008b) and Teulé et al. (2009)
	N/A	31–112	*E. coli*	Lewis et al. (1996)
MaSp2/Flag	*N. clavipes*	58, 62	*E. coli*	Teulé et al. (2007)
MaSp1 & MaSp2	*L. hesperus*	14	*E. coli*	Hagn et al. (2011)
	N. clavipes	20–56	*E. coli*	Arcidiacono et al. (2002) and Mello et al. (2004)
	N. clavipes	N/A	*B. subtilis*	Fahnestock (1994)
	N. clavipes	55, 67	*E. coli*	Brooks et al. (2008a)
	N. clavipes	15–41	*E. coli*	Prince et al. (1995)
	N. clavipes	65–163	*E. coli*	Fahnestock and Irwin (1997)
ADF3, ADF4	*A. diadematus*	34–106	*E. coli*	Schmidt et al. (2007) and Huemmerich et al. (2004a)
ADF1-ADF4	*A. diadematus*	25–56	*S. typhimurium*	Widmaier et al. (2009) and Widmaier and Voigt (2010)
Flag	*N. clavipes*	N/A	*S. typhimurium*	Widmaier et al. (2009) and Widmaier and Voigt (2010)
	N. clavipes	14–94	*E. coli*	Heim et al. (2010) and Vendrely et al. (2008)
	N. clavipes	25	*E. coli*	Zhou et al. (2001)

10.3.1 Fibers

In principle, several techniques can be applied to produce fibers from solutions of recombinant spider silk proteins. Here a short overview on recent attempts of two prominent techniques, namely wet spinning and biomimetic spinning will be given.

10.3.1.1 Wet Spinning

In wet spinning processes, polymer or protein solutions are extruded into a coagulation bath. The proteins, dissolved in aqueous solution or organic solvents are extruded into water, methanol, isopropanol or acetone (Seidel et al. 1998; Hardy et al. 2008). An overview of approaches to wet-spin recombinant spider silk proteins as well as mechanical properties of the achieved fibers are given in Table 10.3.

Table 10.3 Wet spun fibers from recombinant spider silk protein

Silk types	Origin	MW [kDa] of the protein	Mechanical properties		References
			Tensile strength [MPa]	Elasticity [%]	
Flag, MaSp like fusion proteins	*N. clavipes*	58	28.6 ± 17.2	3.7 ± 1.2	Teulé et al. (2007)
		62	10.2 ± 7.3	1.6 ± 1.0	Teulé et al. (2007)
MaSp1, Ma Spidroin 1 + 2	*N. clavipes*	43, 55	–	–	Arcidiacono et al. (2002)
MaSp2	*A. aurantia*	63	6.6 ± 5.1	1.5 ± 0.3	Brooks et al. (2008b)
		67	1.9 ± 2.4	19.0 ± 2.2	Brooks et al. (2008b)
		71	49.5 ± 7.8	3.6 ± 2.6	Brooks et al. (2008b)
ADF3	*A. diadematus*	60–140	–	43.4 − 59.6	Lazaris et al. (2002)
ADF3	*A. diadematus*	106.3	64.6 ± 26.0	10.8 ± 3.1	Keerl and Scheibel (2012)
MaSp1, MaSp2	*N. clavipes*	59–106	–	–	Lazaris et al. (2002)
MaSp2	*N. clavipes*	31–112	–	–	Lewis et al. (1996)
Ma Spidroin 1 analogs	*N. clavipes*	94	100.0 − 150.0	5.0 − 15.0	Bogush et al. (2009)
Ma Spidroin 2 analogs	*N. madagascariensis*	113	100.0 − 150.0	5.0 − 15.0	Bogush et al. (2009)
Ma Spidrion 1 like fusion proteins	*N. clavipes*	64–126	–	–	Yang et al. (2005))
MaSp1	*N. clavipes*	100–284	508.0 ± 108.0	15.0 ± 5.0	Xia et al. (2010)
MaSp1	*N. clavipes*	46	16.3 ± 6.7	1.5 ± 0.4	An et al. (2011)
		70	35.7 ± 8.4	3.1 ± 1.8	An et al. (2011)
MaSp2, Flag	*N. clavipes*	58	28.4 ± 25.4	1.7 ± 0.6	Teulé et al. (2011)
MaSp1, MaSp2 inspired protein	*N. clavipes*	50	280.0 – 350.0	27.0 – 42.0	Elices et al. (2011)

The majority of the recombinant fibers have been made of MaSp1 and MaSp2 derivatives from *Nephila clavipe*s. The molecular weight of the underlying proteins varied from 31 to 284 kDa with some tendency, but no strict relation between the molecular weight and tensile strength. Evidently, both the tensile strength and elasticity differed between fibers made of different recombinant spider silk proteins for reasons of molecular weight, but also protein sequence, concentration of the spinning solution, fiber diameter and the coagulation bath. There are also large deviations in the mechanical properties of the different fibers investigated in individual spinning approaches, as seen in Table 10.3. Such variability makes it hardly possible to exactly predict the fiber's properties before spinning based on the amino acid sequence and/or the molecular weight. In principle the tensile strength of a fiber rises with decreasing diameter (Teulé et al. 2011) and increasing molecular weight of the protein (Xia et al. 2010), reaching a plateau above a specific protein size. Importantly, if compared to the mechanical properties of natural spider silk fibers, all man-made fibers show less mechanical stability, independent of the fiber diameter (Seidel et al. 2000). The reason for this finding could be related to molecular self-assembly of the spidroins. Therefore, a detailed analysis of this assembly is necessary, which is one basis of biomimetic spinning.

10.3.1.2 Biomimetic Spinning

Biomimetic spinning implements all factors that are important in the natural spinning process including molecular self-assembly of the spidroins. The main factors needed for a transition of a soluble state of the spidroins in the spinning dope into a solid fiber during natural spinning are a change in pH, ion exchange and internal water removal. In addition to chemical processes, extensional and shear forces in the duct are necessary to solidify the fiber (Fig. 10.2). Under storage conditions at pH 7, spidroins form micellar-like structures with liquid-crystalline properties to prevent aggregation (Knight and Vollrath 1999; Hu et al. 2007), whereby the terminal domains play a key role (Askarieh et al. 2010; Exler et al. 2007). Along the spinning duct the pH drops from around 7.4 to 6 and shear forces increase. Studies showed, that recombinant spidroins with NRC domains assemble into fibers while recombinant proteins without NRC domains only unspecifically aggregate (Hagn et al. 2010; Eisoldt et al. 2010; Rammensee et al. 2008). Further, the NRC domain has been shown to be important for pre-orientation of the core domains structure (Askarieh et al. 2010; Exler et al. 2007; Eisoldt et al. 2010).

Such knowledge has been used to develop microfluidic devices mimicking the geometry of silk glands, and which allowed controlling the chemical and mechanical parameters necessary to produce silk fibers.

Rammensee et al. presented a microfluidic device which allowed to assemble recombinant spidroins in aqueous solution using solely the natural triggers (Rammensee et al. 2008). Next generation microfluidic devices allowed to produce functional spider silk fibers with predictable diameter (Kinahan et al. 2011).

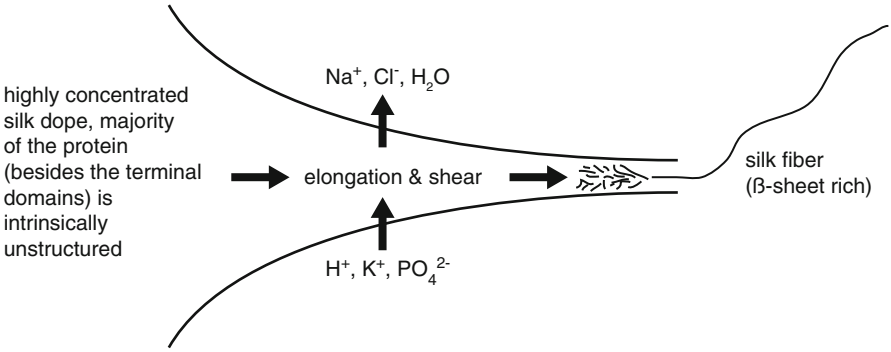

Fig. 10.2 Schematic overview of the parameters critical for the natural spinning process of spider dragline silk. During this process, the pH is lowered and phosphate and potassium ions are pumped into the silk dope, while water and chloride are extracted from the dope. Mechanical stress induces the formation of a silk fiber with high β-sheet content

The fiber diameter can be controlled by the flow rate of the protein solution within the microfluidic channel. The velocity along the silk gland of spiders rises due to a steady decrease in radius (Breslauer et al. 2009). Different spinning speeds have also a significant effect on the properties of the resulting fibers (Vollrath et al. 2001), likely because of higher molecular alignment caused by stretching of the fiber. *In vitro*, higher flow rates than in natural processes are needed based on less concentrated protein solutions in comparison to nature where increased concentration and thereby viscosity leads to fiber formation at lower elongational flow rates (Rammensee et al. 2008). Although microfluidic devices provide a simple method to produce uniformly sized fibers, there are still problems to produce endless and consistent fibers with properties similar to that of the natural blueprint.

10.3.2 Non-woven Meshes Made of Recombinant Spider Silk Proteins

Electrospinning of biopolymers is a cost effective and easy way to produce non-woven meshes with high inter-fiber spacing and fiber diameters ranging from micrometers down to a few nanometers (Frenot and Chronakis 2003). The advantage of electrospinning is the low amount of polymer solution needed to generate continuous fibers (Baumgarten 1971). If an electric field is applied to a pendant droplet of polymer solution a taylor conus is formed, and a polymer jet is created directing towards the counter electrode. The solvent evaporates before reaching the collector plate, and dried fibers can be collected e.g. as a non-woven mat (Huang et al. 2003). The fiber diameter can be controlled by the viscosity, the concentration of the polymer solution, the salt content, the surface tension of the solvent, the distance to the counter electrode, as well as the polymer itself

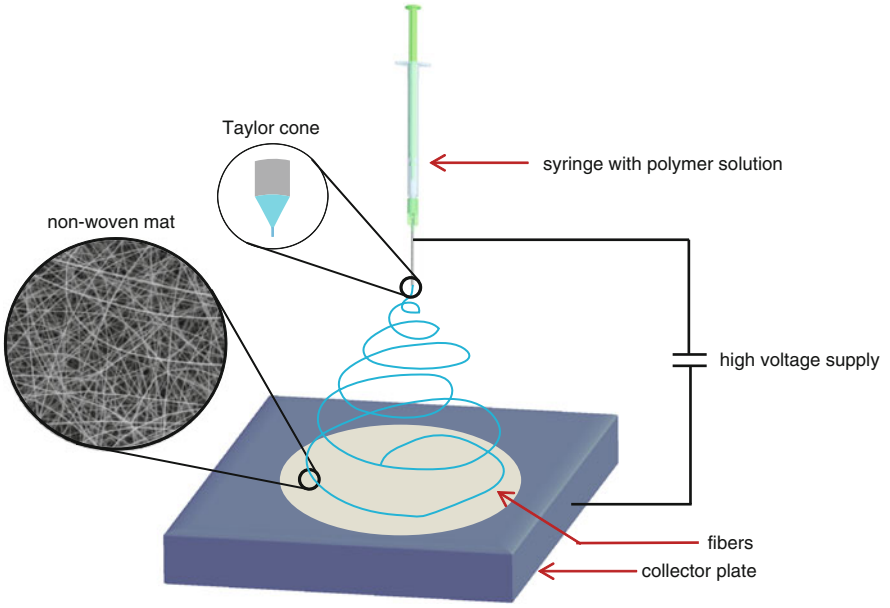

Fig. 10.3 Scheme of an electrospinning setup. An electric field is applied to a pendant droplet of polymer solution leading to a taylor conus, from which a polymer jet emerges. The solvent evaporates before reaching the collector plate, and dried fibers can be collected e.g. as a non-woven mat (Huang et al. 2003)

(molecular weight, molecular weight distribution, solubility, and glass transition temperature) (Heikkila and Harlin 2008; Greiner and Wendorff 2007). Furthermore, the surrounding temperature and the relative humidity can significantly influence fiber formation (Fig. 10.3).

To guarantee the continuity of the fibers, the concentration of the silk solution must be high enough to permit the entanglement between the silk molecules. Interaction between the solvent and the silk molecules, as well as their molecular weight, will influence the minimal concentration needed (Chengjie et al. 2009). Depending on the collector set up, single fibers or non-woven meshes can be achieved, the later showing a high surface area to volume ratio and a high potential for applications in e.g. tissue engineering and wound healing.

Several groups have electrospun different silk types, including regenerated *B. mori* silk, regenerated *N. clavipes* dragline silk and silk-like polymers from organic solvents like formic acid (FA), Hexafluoroisopropanol (HFIP) or Hexafluoracetone (HFA) (Buchko et al. 1999; Jin et al. 2002; Zarkoob et al. 2004). Only a few electrospinning setups employed recombinant spider silk proteins as depicted below.

The recombinant spider silk protein eADF4(C16) (based on the dragline silk protein ADF4 of *A. diadematus*) was dissolved in HFIP at concentrations from 4 % (w/v) to 24 % (w/v). Below 8 % (w/v) nanoparticles were obtained (akin to electro-

spraying), while homogeneous fibers could only be produced at concentrations above 16 % (w/v). With increasing protein concentration the fibers diameter increased from 150 to 700 nm (Leal-Egana et al. 2012). All as-spun eADF4(C16) fibers from HFIP were water soluble and predominantly consisted of random coil and α-helical structures. Since in most applications water stable fibers are necessary, the non-woven meshes can be post-treated with methanol vapor to induce the formation of β-sheet structures (Leal-Egana et al. 2012; Lang et al. 2013). Another way to promote β-sheet formation in non-woven meshes is temperature or humidity annealing (Wang et al. 2006; Zarkoob et al. 2004).

Cell culture experiments with BALB/3T3 mouse fibroblasts on post-treated eADF4(C16) non-woven meshes showed that cell adhesion and proliferation were strictly dependent on the diameter of the individual fibers. With increasing fiber diameter cells adhere much better to the meshes, and their doubling time decreases while their proliferation rate increases. One explanation for this phenomena is that the larger the fiber diameter, the larger the spacing between the fibers, making it easier for lamellipodia and filopodia to protrude (Leal-Egana et al. 2012).

Bini et al. created two engineered proteins based on the consensus sequence derived from MaSp 1 of *Nephila calvipes*. One of the proteins was further fused with an RGD motif to enhance cell interactions. Non-woven meshes comprising these proteins were spun from HFIP solutions, and the obtained fiber diameters ranged from 50 to 250 nm with an average diameter of 100 nm. To increase the β-sheet content non-woven meshes were post-treated with methanol (Bini et al. 2006) (cf. Sect. 10.4.1).

To generate organic–inorganic composite non-woven scaffolds, an R5 peptide (derived from the repetitive motif of silaffin proteins) was fused to *N. clavipes* spider dragline silk protein, either in presence or absence of an additional RGD motif (Foo et al. 2006). Fibers of the chimeric spider silk-silaffin proteins were spun from HFIP. Incubation of these non-woven meshes with silicic acid solution induced silica sphere formation on the non-woven mats with diameters ranging from 200 to 400 nm. Since the non-woven meshes were not post-treated, the fibers fused upon incubation in silicic acid. Contrarily, when non-woven meshes were treated with methanol before silification, silica nanospheres were sparsely observed. Silification reactions during electrospinning (concurrent processing) resulted in a non-uniform coating of the fibers but no particle formation (Foo et al. 2006).

10.4 Modification of Recombinant Silk Proteins

One advantage of recombinant spider silk proteins is the ease of genetic modification which allows the direct incorporation of functional groups into the silk proteins (as already depicted in some examples in Sect. 10.3.) Alternatively, chemical modification of distinct naturally occurring or artificially introduced specific amino acid side chains is feasible.

Table 10.4 Chimeric spider silk proteins for diverse biomedical applications

Fusion protein	Application	References
Silk + silicifying peptides	Scaffolds for bone regeneration	Foo et al. (2006), Mieszawska et al. (2010), and Belton et al. (2012)
Silk + dentin matrix protein	Scaffolds for bone regeneration	Huang et al. (2007)
Silk + RGD peptides	Biomedical applications	Bini et al. (2006), Morgan et al. (2008), Wohlrab et al. (2012), and Bauer et al. (2013)
Silk + poly(L-lysine)	Drug delivery	Numata et al. (2009, 2012)
Silk + poly(L-lysine) + cell penetrating peptide	Drug delivery	Numata and Kaplan (2010)
Silk + antimicrobial domain	Tissue engineering	Currie et al. (2011) and Gomes et al. (2011)

10.4.1 Genetic Engineering

Genetic engineering of silk genes allows to incorporate either individual amino acids, or even peptide sequences that enable enhanced cell adhesion or improved solubility (Table 10.4). In the following, several attempts to modify silk proteins for specific applications are summarized.

10.4.1.1 Biomineralisation

Biosilica architectures in diatoms are produced under ambient conditions (aqueous solution, neutral pH and low temperatures). *In vitro*, the R5 peptide (derived from the repetitive motif of the silaffin protein of *Cylindrotheca fusiformis*) regulates and induces silica formation under similar conditions. To generate scaffolds for bone regeneration, the R5 sequence was genetically fused to an RGD containing *N. clavipes* spider dragline silk protein (as mentioned in Sect. 10.3.) (Foo et al. 2006). Besides fibers, the resulting chimeric silk-silica proteins were processed into films. By treating such films with silicic acid solution, the R5 peptide induces biomineralization on the surface (Foo et al. 2006). Osteogenic differentiation was analyzed culturing human mesenchymal stem cells (hMSCs) on such silk-silica protein films. The bound silica influenced osteogenic gene expression with upregulation of alkaline phosphatase (ALP), bone sialoprotein (BSP), and collagen type 1 (Col 1). Calcium deposits on silk-silica films further indicated enhanced osteogensis (Mieszawska et al. 2010).

Belton et al. determined the silica condensation using a range of silicifying peptides (R5: SSKKSGSYSGSKGSKRRIL; A1: SGSKGSKRRIL; Si4-1: MSPHPHPRHHHT, and repeats thereof) fused to the N-terminus of a recombinant *N. clavipes* spider dragline silk protein. The authors determined a strict relationship

between silk solution properties (e.g. pH of the solution) and silica deposition, leading to silica silk chimera material formation (Belton et al. 2012).

Another approach for using silk scaffolds in bone formation was investigated by Huang et al., producing a chimeric protein based on the MaSp1 of *N. clavipes* and dentin matrix protein 1 which is involved in the nucleation and crystallization of hydroxyapatite. The recombinantly produced protein was processed into films, which showed no structural differences in comparison to films of non-modified silk. Incubation of processed films in simulated body fluids induced the growth of hydroxyapatite crystals on silk films with the fused dentin matrix protein 1, indicating their potential for applications in bone tissue engineering (Huang et al. 2007).

10.4.1.2 Cell Adhesion

For biomedical applications the interaction of a material's surface with cells or tissue is highly important. One strategy to improve cell adhesion is to change the surface topography of a material (Leal-Egana et al. 2012; Leal-Egana and Scheibel 2012; Bauer et al. 2013). Another one is modification of the silk proteins with cell adhesive peptides. The recombinant spider silk protein eADF4(C16) was genetically modified with the linear cell adhesion sequence GRGDSPG. The RGD-modified protein was successfully processed into films, and cell adhesion and proliferation of mouse fibroblasts (BALB/3T3) was investigated thereon. In comparison to unmodified spider silk films, cells on RGD-modified films showed improved adhesion and proliferation (Wohlrab et al. 2012).

Bini et al. combined the consensus sequence derived from MaSp1 of *N. clavipes* with the cell binding motif RGD. The modified silk was processed into films and fibers, which were successfully used for culturing human bone marrow stromal cells (hMSCs). In comparison to the tissue culture plastic, silk surfaces showed increased calcium deposition, but surprisingly no impact on cell differentiation was observed (Bini et al. 2006). Since cell binding is dependent on the surface density of RGD, Morgan et al. blended RGD modified recombinant spidroin with *B. mori* silk in different ratios (10:90, 30:70, 50:50, 70:30, 90:10 RGD-spidroin: silk fibroin) to adjust the RGD concentration exposed on the silk film surface. Strikingly, the proliferation and differentiation of pre-osteoblasts (MC3T3-E1) was indistinguishable between the various blends (Morgan et al. 2008).

10.4.1.3 Gene Delivery

Engineered block copolymers of spider silk with poly(L-lysine) domains were investigated as gene delivery systems. The silk-poly(L-lysine) copolymer self-assembles into complexes with plasmid DNA (pDNA) through electrostatic interactions. The resulting particles delivered genes into human embryonic kidney cells (HEK) (Numata et al. 2009). To increase the delivery efficiency, incorportation of

cell penetrating peptids (CPPs), like ppTG1, into silk hybrids was investigated. CPPs which are known to be amphipathic and positively charged (Zorko and Langel 2005) are able to deliver large-cargo molecules into cells (Madani et al. 2011). Complexes of the silk-poly(L-lysine)-ppTG1 protein with pDNA showed a transfection efficiency comparable to the transfection reagent Lipofectamine 2000 (Numata and Kaplan 2010). In another study the tumor-homing peptide (THP) was fused to a silk-poly(L-lysine) block copolymer. The pDNA complex of silk-poly(L-lysine)-THP block copolymers showed a significantly enhanced targeting of specific tumor cells (Numata et al. 2011, 2012).

10.4.1.4 Antimicrobial Silk

Silver containing silk materials could be used in applications in which antimicrobial activity is needed. Therefore, Currie et al. fused a silver binding peptide to a recombinant spider dragline silk protein derived from MaSp1 of *N. clavipes*. The resulting protein nucleated Ag ions from a silver nitrate solution (Currie et al. 2011). Regarding antimicrobial activity, Gomes et al. fused the consensus sequence derived from the MaSp1 of *N. clavipes* with three different antimicrobial peptides (the human antimicrobial peptides human neutrophil defensin 2 (HNP-2), human neutrophil defensin 4 (HNP-4) and hepcidin). The recombinantly produced chimeric proteins were processed into films, and it was demonstrated that the silk domain retained its self-assembly properties. The antimicrobial activity against *E. coli* and *S. aureus* was analyzed, and the microbial activity was demonstrated. Furthermore, cell studies with a human osteosarcoma cell line (SaOs-2) demonstrated the compatibility of these films with mammalian cells (Gomes et al. 2011).

10.4.1.5 Others

To control the solubility of spider silk proteins Winkler et al. incorporated methionine residues next to polyalanine sequences, found in the dragline silk of *N. clavipes*, to trigger β-sheet formation. This methionine residues can work as redox triggers (Winkler et al. 1999). The oxidation to the sulfoxide state with phenacyl bromide yields a water-soluble protein and prevents the formation of β-sheets by disrupting the hydrophobic interactions between the overlaying sheets. Reduction with β-mercaptoethanol, in contrast, triggers self-assembly into β-sheets, without disturbing the general macromolecular assembly behavior (Valluzzi et al. 1999; Szela et al. 2000).

To control the β-sheet content, enzymatic phosphorylation and dephosphorylation reactions were performed with genetically engineered spider dragline silk proteins. By introducing charged phosphate groups using cyclic AMP-dependent protein kinase, hydrophobic interactions between the β-sheets were prevented, and thus solubility of the proteins increased. Concomitantly, dephosphorylation with calf intestinal alkaline phosphatase induced self-assembly and β-sheet formation (Winkler et al. 2000).

In addition to functional peptide sequences, individual amino acid residues with chemically specific side chains (such as thiols of cysteine residues) can be incorporated in engineered spider silk proteins. Due to the fact that thiol side chains of cysteines are ideally suited for modifications at neutral pH, a single cysteine was introduced in eADF4(C16) (Spiess et al. 2010). In comparison to the cysteine free variant, no differences concerning protein structure or assembly were detectable in solution and in the films. It could be shown that target molecules like maleimide-conjugated fluorescein were successfully coupled to the cysteine-modified silk proteins in processed films as well as in solution.

10.4.2 Chemical Functionalization

Chemical modification of silk proteins is an alternative route towards the functionalization of silk materials. Due to the fact that most chemical reactions occur in the presence of other functional groups, there must be a high selectivity thereof. Further, the coupling should in the best case take place at physiological conditions (Sletten and Bertozzi 2009). The functionalization of silk proteins is often much easier than that of globular proteins, since silk proteins are mostly intrinsically unfolded in aqueous solution. Typically, modifications of amino acid side chains are achieved by modifications using electrophilic or nucleophilic groups (Carrico 2008). Most recombinantly produced spidroins comprise non-reactive glycine and alanine residues and lack amino acids residues with functional groups like lysines or cysteines. Nevertheless glutamic acid, aspartic acid as well as tyrosine residues allow specific chemical modifications of spidroins (Vendrely and Scheibel 2007).

Modification of recombinant spider silk proteins is possible in solution and after processing into different morphologies (Fig. 10.4a). For example small organic molecules as well as biological macromolecules like enzymes can be immobilized on eADF4(C16) films by modification of glutamic acid residues. After activation of the carboxyl groups (one per repetitive unit in addition to the C-terminus) with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysulfosuccinimide (EDC/NHS), the enzyme β-galactosidase was efficiently coupled (Huemmerich et al. 2006). The activity of the enzyme after coupling was demonstrated using the specific substrate 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal). Employing an ethylenediamine linker after EDC/NHS activation allowed coupling of fluorescein-5-isothiocyanat (FITC) to the eADF4(C16) film's surface.

To create a single chemical bond between the recombinant spider silk protein eADF4(C16) and a target, one way is the modification of the N-terminus (since eADF4(C16) does not contain any other primary amines) (Schacht and Scheibel 2011). The one-step reaction of N-hydroxysuccinimde (NHS)-activated carboxylic acid targets with primary amines is, however, limited by a low specificity (Thordarson et al. 2006). Due to the fact that thiol side chains of cysteine residues are ideally suited for modifications at neutral pH, a single cysteine residue was introduced in eADF4(C16) (Spiess et al. 2010) (Fig. 10.4b, see also Sect. 10.4.1.5).

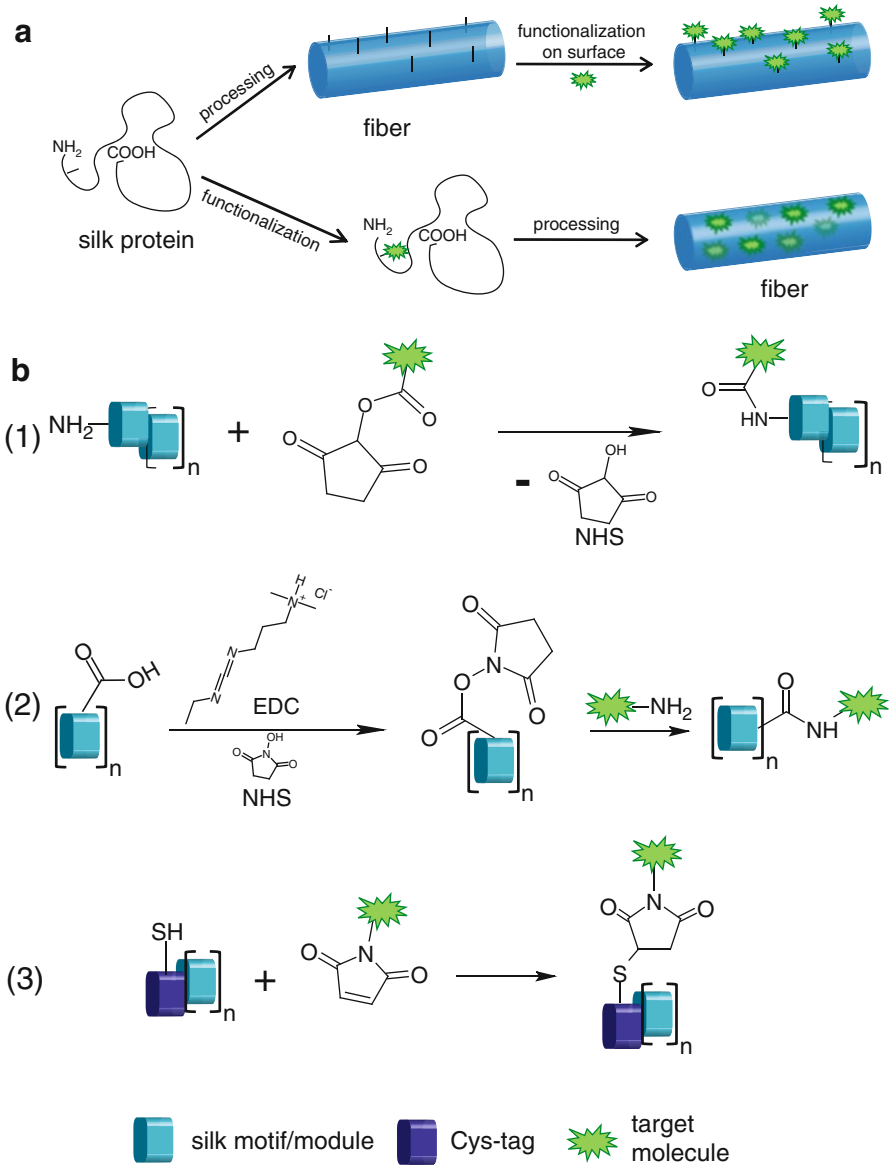

Fig. 10.4 Chemical modification of engineered spider silk proteins; (**a**) Two routes of functionalization of silk fibers can be achieved: processing of the fibers followed by chemical modification or chemical functionalization in aqueous solution followed by fiber production; (**b**) Modification of recombinant silk proteins with N-hydroxysuccinimde (NHS)-activated carboxylic acid targets at primary amines (*1*) or functionalization of terminal carboxyl groups and glutamic acid residues with EDC/NHS activation followed by functionalization with different primary amines (*2*) (Huemmerich et al. 2006) or at thiol groups in cysteine modified variants through maleimide chemistry (*3*) (Spiess et al. 2010; Humenik et al. 2011; Schacht and Scheibel 2011)

Thiol modification was performed using maleimide, which reacts selectively and quantitatively in a single step procedure with thiol groups forming a stable thioether bond (Partis et al. 1983; Heitz et al. 1968). It has been shown that after eADF4(C16) film formation, target molecules like maleimide-conjugated fluorescein, monomaleimido-nanogold particles and β-galactosidase (in combination with NHS-PEO_{12}-Maleimide crosslinker) could be successfully coupled (Spiess et al. 2010). Since there are no side-reactive complications, like in the case of carbodiimide activation (glutamic acid residues), the reaction was further used to couple a maleimide modified cyclic RGD peptide (Wohlrab et al. 2012).

10.4.3 Silk-Polymer Hybrids

Silk peptides can be combined with synthetic polymers like poly(ethylene glycol) (PEG) or poly(isoprene) to achieve new functionalities (Zhou et al. 2006; Rathore and Sogah 2001). Rathore and Sogah designed a *N. clavipes*- inspired block-copolymer by selective replacement of the amorphous peptide domain with flexible non-peptidic poly(ethylene glycol) (PEG) blocks, while retaining the poly(alanine) sequences (Rathore and Sogah 2001). The silk-polymer hybrids were synthesized with varying chain length of the poly(alanines) blocks via prefabricated blocks which were linked block by block (Lego method) (Winningham and Sogah 1997; Rathore and Sogah 2001). Despite the replacement, the silk peptide blocks achieved β-sheet conformation in the resulting polymer. Next, the mechanical properties of silk-polymer films and fibers were determined. Compared with films made of a *B. mori*-inspired block copolymer, the spider silk-inspired analogue films were tougher and stronger. Furthermore, increasing the length of the poly(alanine) block resulted in increased elastic modulus and tensile strength. As a result of the higher stiffness, the elongation at break decreased with increasing length of the poly(alanine) block (Rathore and Sogah 2001).

Since oligo- and polythiophenes are an interesting class of (semi)conducting materials, Klok et al. established the synthesis of diblock oligomers with oligo(3-alkylthiophene) (HT-O3AT) and the silk inspired pentapeptide (Gly-Ala-Gly-Ala-Gly) via solid phase acylation of the resin-bound pentapeptide for electronic applications. In addition to β-alkylated oligothiophenes which usually form organized lamellar assemblies, silk peptide conjugate assembly is driven by directed hydrogen bonding interactions (Klok et al. 2004).

In one approach a low-molecular-weight gelator (LMWGs) based on the silk-like tetrapeptide Gly-Ala-Gly-Ala was designed. To introduce additional van der Waals interactions and to regulate the solubility, apolar alkyl tails (C_{12}) were added as terminal groups (Escuder and Miravet 2006). Gels with a β-sheet fibril network were obtained in several organic solvents like tetrahydrofuran, chloroform, cyclohexane or toluene. The fibrils (less than 20 nm) assembled into a network of bundles with a length of several micrometers and a width of ∼100 nm (Escuder and Miravet 2006). By varying the alkyl chain length (from C_3 to C_{12}) or by adding aromatic

groups (phenyl or 4-nitrophenyl), the gelation behavior in different polar and apolar solvents could be controlled. For example, only compounds with similar alkyl chains on both ends formed gels in acetone, whereas compounds with dissimilar alkyl chains were not able to form gels (Iqbal et al. 2008).

10.5 Outlook

Spider silks are known for their outstanding mechanical properties and their biocompatibility since ages. Over the last decades, scientists developed several approaches to produce spider silk proteins recombinantly. Although major improvements have been made, certain questions still remain, concerning assembly, solubility and storage of spider silk proteins. Nevertheless, the possibility to process spider silk proteins into various morphologies together with the ability to produce different spider silk chimera and spider silk inspired polymers will allow various applications in medical and technical fields.

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References

An B, Hinman MB, Holland GP, Yarger JL, Lewis RV (2011) Inducing beta-sheets formation in synthetic spider silk fibers by aqueous post-spin stretching. Biomacromolecules 12(6):2375–2381. doi:10.1021/bm200463e

Arcidiacono S, Mello C, Kaplan D, Cheley S, Bayley H (1998) Purification and characterization of recombinant spider silk expressed in Escherichia coli. Appl Microbiol Biotechnol 49(1):31–38. doi:10.1007/s002530051133

Arcidiacono S, Mello CM, Butler M, Welsh E, Soares JW, Allen A, Ziegler D, Laue T, Chase S (2002) Aqueous processing and fiber spinning of recombinant spider silks. Macromolecules 35(4):1262–1266. doi:10.1021/ma011471o

Askarieh G, Hedhammar M, Nordling K, Saenz A, Casals C, Rising A, Johansson J, Knight SD (2010) Self-assembly of spider silk proteins is controlled by a pH-sensitive relay. Nature 465(7295):236–238. doi:10.1038/nature08962

Ayoub NA, Garb JE, Tinghitella RM, Collin MA, Hayashi CY (2007) Blueprint for a high-performance biomaterial: full-length spider dragline silk genes. PLoS One 2(6):e514. doi:10.1371/journal.pone.0000514

Bauer F, Wohlrab S, Scheibel T (2013) Controllable cell adhesion, growth and orientation on layered silk protein films. Biomater Sci. doi:10.1039/C3BM60114E

Baumgarten PK (1971) Electrostatic spinning of acrylic microfibers. J Colloid Interface Sci 36(1):71–79. doi:10.1016/0021-9797(71)90241-4

Belton DJ, Mieszawska AJ, Currie HA, Kaplan DL, Perry CC (2012) Silk-silica composites from genetically engineered chimeric proteins: materials properties correlate with silica condensation rate and colloidal stability of the proteins in aqueous solution. Langmuir 28(9):4373–4381. doi:10.1021/La205084z

Bini E, Foo CW, Huang J, Karageorgiou V, Kitchel B, Kaplan DL (2006) RGD-functionalized bioengineered spider dragline silk biomaterial. Biomacromolecules 7(11):3139–3145. doi:10.1021/bm0607877

Bogush VG, Sokolova OS, Davydova LI, Klinov DV, Sidoruk KV, Esipova NG, Neretina TV, Orchanskyi IA, Makeev VY, Tumanyan VG, Shaitan KV, Debabov VG, Kirpichnikov MP (2009) A novel model system for design of biomaterials based on recombinant analogs of spider silk proteins. J Neuroimmune Pharmacol 4(1):17–27. doi:10.1007/s11481-008-9129-z

Breslauer DN, Lee LP, Muller SJ (2009) Simulation of flow in the silk gland. Biomacromolecules 10(1):49–57. doi:10.1021/Bm800752x

Brooks AE, Nelson SR, Jones JA, Koenig C, Hinman M, Stricker S, Lewis RV (2008a) Distinct contributions of model MaSp1 and MaSp2 like peptides to the mechanical properties of synthetic major ampullate silk fibers as revealed in silico. Nanotechnol Sci Appl 1:9–16. doi:10.2147/NSA.S3961

Brooks AE, Stricker SM, Joshi SB, Kamerzell TJ, Middaugh CR, Lewis RV (2008b) Properties of synthetic spider silk fibers based on *Argiope aurantia* MaSp2. Biomacromolecules 9(6):1506–1510. doi:10.1021/bm701124p

Buchko CJ, Chen LC, Shen Y, Martin DC (1999) Processing and microstructural characterization of porous biocompatible protein polymer thin films. Polymer 40(26):7397–7407. doi:10.1016/S0032-3861(98)00866-0

Carrico IS (2008) Chemoselective modification of proteins: hitting the target. Chem Soc Rev 37(7):1423–1431. doi:10.1039/B703364h

Chengjie F, Zhengzhong S, Vollrath F (2009) Animal silks: their structures, properties and artificial production. Chem Commun 43:6515–6529. doi:10.1039/B911049F

Colgin MA, Lewis RV (1998) Spider minor ampullate silk proteins contain new repetitive sequences and highly conserved non-silk-like "spacer regions". Protein Sci 7(3):667–672. doi:10.1002/pro.5560070315

Currie HA, Deschaume O, Naik RR, Perry CC, Kaplan DL (2011) Genetically engineered chimeric silk-silver binding proteins. Adv Funct Mater 21(15):2889–2895. doi:10.1002/adfm.201100249

Eisoldt L, Hardy JG, Heim M, Scheibel TR (2010) The role of salt and shear on the storage and assembly of spider silk proteins. J Struct Biol 170(2):413–419. doi:10.1016/j.jsb.2009.12.027

Eisoldt L, Scheibel T, Smith A (2011) Decoding the secrets of spider silk. Mater Today 14(3):80–86. doi:10.1016/S1369-7021(11)70057-8

Elices M, Guinea GV, Plaza GR, Karatzas C, Riekel C, Agulló-Rueda F, Daza R, Pérez-Rigueiro J (2011) Bioinspired fibers follow the track of natural spider silk. Macromolecules 44(5):1166–1176. doi:10.1021/ma102291m

Escuder B, Miravet JF (2006) Silk-inspired low-molecular-weight organogelator. Langmuir 22(18):7793–7797. doi:10.1021/La060499w

Exler JH, Hummerich D, Scheibel T (2007) The amphiphilic properties of spider silks are important for spinning. Angew Chem Int Ed 46(19):3559–3562. doi:10.1002/anie.200604718

Fahnestock S (1994) Novel, recombinantly produced spider silk analogs. USA Patent WO 94/29450, 22 Dec 1994

Fahnestock SR, Irwin SL (1997) Synthetic spider dragline silk proteins and their production in Escherichia coli. Appl Microbiol Biotechnol 47(1):23–32. doi:10.1007/s002530050883

Foo CWP, Patwardhan SV, Belton DJ, Kitchel B, Anastasiades D, Huang J, Naik RR, Perry CC, Kaplan DL (2006) Novel nanocomposites from spider silk-silica fusion (chimeric) proteins. Proc Natl Acad Sci USA 103(25):9428–9433. doi:10.1073/pnas.0601096103

Frenot A, Chronakis IS (2003) Polymer nanofibers assembled by electrospinning. Curr Opin Colloid Interface Sci 8(1):64–75. doi:10.1016/S1359-0294(03)00004-9

Fukushima Y (1998) Genetically engineered syntheses of tandem repetitive polypeptides consisting of glycine-rich sequence of spider dragline silk. Biopolymers 45(4):269–279. doi:10.1002/(SICI)1097-0282(19980405)4

Garb JE, Ayoub NA, Hayashi CY (2010) Untangling spider silk evolution with spidroin terminal domains. BMC Evol Biol 10:243. doi:10.1186/1471-2148-10-243

Geurts P, Zhao L, Hsia Y, Gnesa E, Tang S, Jeffery F, Mattina CL, Franz A, Vierra C (2010) Synthetic spider silk fibers spun from pyriform spidroin 2, a glue silk protein discovered in orb-weaving spider attachment discs. Biomacromolecules 11(12):3495–3503. doi:10.1021/bm101002w

Gnesa E, Hsia Y, Yarger JL, Weber W, Lin-Cereghino J, Lin-Cereghino G, Tang S, Agari K, Vierra C (2012) Conserved C-terminal domain of spider tubuliform spidroin 1 contributes to extensibility in synthetic fibers. Biomacromolecules 13(2):304–312. doi:10.1021/bm201262n

Gomes SC, Leonor IB, Mano JF, Reis RL, Kaplan DL (2011) Antimicrobial functionalized genetically engineered spider silk. Biomaterials 32(18):4255–4266. doi:10.1016/j.biomaterials.2011.02.040

Gosline JM, Guerette PA, Ortlepp CS, Savage KN (1999) The mechanical design of spider silks: from fibroin sequence to mechanical function. J Exp Biol 202(23):3295–3303

Greiner A, Wendorff JH (2007) Electrospinning: a fascinating method for the preparation of ultrathin fibres. Angew Chem Int Ed 46(30):5670–5703. doi:10.1002/anie.200604646

Grip S, Rising A, Nimmervoll H, Storckenfeldt E, McQueen-Mason SJ, Pouchkina-Stantcheva N, Vollrath F, Engström W, Fernandez-Arias A (2006) Transient expression of a major ampullate spidroin 1 gene fragment from *Euprosthenops* sp. in mammalian cells. Cancer Genomics Proteomics 3(2):83–87

Guerette PA, Ginzinger DG, Weber BHF, Gosline JM (1996) Silk properties determined by gland-specific expression of a spider fibroin gene family. Science 272(5258):112–115. doi:10.1126/science.272.5258.112

Hagn F, Eisoldt L, Hardy JG, Vendrely C, Coles M, Scheibel T, Kessler H (2010) A highly conserved spider silk domain acts as a molecular switch that controls fibre assembly. Nature 465(7295):239–242. doi:10.1038/nature08936

Hagn F, Thamm C, Scheibel T, Kessler H (2011) pH-dependent dimerization and salt-dependent stabilization of the N-terminal domain of spider dragline silk–implications for fiber formation. Angew Chem Int Ed 50(1):310–313. doi:10.1002/anie.201003795

Hardy JG, Romer LM, Scheibel TR (2008) Polymeric materials based on silk proteins. Polymer 49(20):4309–4327. doi:10.1016/j.polymer.2008.08.006

Hayashi CY, Blackledge TA, Lewis RV (2004) Molecular and mechanical characterization of aciniform silk: uniformity of iterated sequence modules in a novel member of the spider silk fibroin gene family. Mol Biol Evol 21(10):1950–1959. doi:10.1093/molbev/msh204

Hedhammar M, Rising A, Grip S, Martinez AS, Nordling K, Casals C, Stark M, Johansson J (2008) Structural properties of recombinant nonrepetitive and repetitive parts of major ampullate spidroin 1 from Euprosthenops australis: implications for fiber formation. Biochemistry 47(11):3407–3417. doi:10.1021/bi702432y

Heikkila P, Harlin A (2008) Parameter study of electrospinning of polyamide-6. Eur Polym J 44(10):3067–3079. doi:10.1016/j.eurpolymj.2008.06.032

Heim M, Keerl D, Scheibel T (2009) Spider silk: from soluble protein to extraordinary fiber. Angew Chem Int Ed 48(20):3584–3596. doi:10.1002/anie.200803341

Heim M, Ackerschott CB, Scheibel T (2010) Characterization of recombinantly produced spider flagelliform silk domains. J Struct Biol 170(2):420–425. doi:10.1016/j.jsb.2009.12.025

Heitz JR, Anderson CD, Anderson BM (1968) Inactivation of yeast alcohol dehydrogenase by N-alkylmaleimides. Arch Biochem Biophys 127(1–3):627–636. doi:10.1016/0003-9861(68)90271-3

Hu XY, Yuan J, Wang XD, Vasanthavada K, Falick AM, Jones PR, La Mattina C, Vierra CA (2007) Analysis of aqueous glue coating proteins on the silk fibers of the cob weaver, Latrodectus hesperus. Biochemistry 46(11):3294–3303. doi:10.1021/bi602507e

Huang ZM, Zhang YZ, Kotaki M, Ramakrishna S (2003) A review on polymer nanofibers by electrospinning and their applications in nanocomposites. Composites Sci Technol 63(15):2223–2253. doi:10.1016/S0266-3538(03)00178-7

Huang J, Wong C, George A, Kaplan DL (2007) The effect of genetically engineered spider silk-dentin matrix protein 1 chimeric protein on hydroxyapatite nucleation. Biomaterials 28(14):2358–2367. doi:10.1016/j.biomaterials.2006.11.021

Huemmerich D, Helsen CW, Quedzuweit S, Oschmann J, Rudolph R, Scheibel T (2004a) Primary structure elements of spider dragline silks and their contribution to protein solubility. Biochemistry 43(42):13604–13612. doi:10.1021/Bi048983q

Huemmerich D, Scheibel T, Vollrath F, Cohen S, Gat U, Ittah S (2004b) Novel assembly properties of recombinant spider dragline silk proteins. Curr Biol 14(22):2070–2074. doi:10.1016/j.cub.2004.11.005

Huemmerich D, Slotta U, Scheibel T (2006) Processing and modification of films made from recombinant spider silk proteins. Appl Phys A: Mater Sci Process 82(2):219–222. doi:10.1007/s00339-005-3428-5

Humenik M, Smith AM, Scheibel T (2011) Recombinant spider silks—biopolymers with potential for future applications. Polymers 3(1):640–661. doi:10.3390/polym3010640

Iqbal S, Miravet JF, Escuder B (2008) Biomimetic self-assembly of tetrapeptides into fibrillar networks and organogels. Eur J Org Chem 27:4580–4590. doi:10.1002/ejoc.200800547

Ittah S, Cohen S, Garty S, Cohn D, Gat U (2006) An essential role for the C-terminal domain of a dragline spider silk protein in directing fiber formation. Biomacromolecules 7(6):1790–1795. doi:10.1021/bm060120k

Jin HJ, Fridrikh SV, Rutledge GC, Kaplan DL (2002) Electrospinning *Bombyx mori* silk with poly(ethylene oxide). Biomacromolecules 3(6):1233–1239. doi:10.1021/Bm025581u

Karatzas CN, Turner JD, Karatzas A-L (1999) Production of biofilaments in transgenic animals. Canada Patent WO 99/47661

Keerl D, Scheibel T (2012) Characterization of natural and biomimetic spider silk fibers. Bioinspired Biomim Nanobiomaterials 1(2):83–94. doi:10.1680/bbn.11.00016

Kinahan ME, Filippidi E, Koster S, Hu X, Evans HM, Pfohl T, Kaplan DL, Wong J (2011) Tunable silk: using microfluidics to fabricate silk fibers with controllable properties. Biomacromolecules 12(5):1504–1511. doi:10.1021/bm1014624

Klok HA, Rosler A, Gotz G, Mena-Osteritz E, Bauerle P (2004) Synthesis of a silk-inspired peptide oligothiophene conjugate. Org Biomol Chem 2(24):3541–3544. doi:10.1039/B415454a

Knight DP, Vollrath F (1999) Liquid crystals and flow elongation in a spider's silk production line. Proc Biol Sci 266(1418):519–523. doi:10.1098/rspb.1999.0667

Lang G, Jokisch S, Scheibel T (2013) Air filter devices including nonwoven meshes of electrospun recombinant spider silk proteins. J Vis Exp 75:e50492. doi:10.3791/50492

Lawrence BA, Vierra CA, Mooref AMF (2004) Molecular and mechanical properties of major ampullate silk of the black widow spider, Latrodectus hesperus. Biomacromolecules 5(3):689–695. doi:10.1021/Bm0342640

Lazaris A, Arcidiacono S, Huang Y, Zhou JF, Duguay F, Chretien N, Welsh EA, Soares JW, Karatzas CN (2002) Spider silk fibers spun from soluble recombinant silk produced in mammalian cells. Science 295(5554):472–476. doi:10.1126/science.1065780

Leal-Egana A, Scheibel T (2012) Interactions of cells with silk surfaces. J Mater Chem 22(29):14330–14336. doi:10.1039/C2jm31174g

Leal-Egana A, Lang G, Mauerer C, Wickinghoff J, Weber M, Geimer S, Scheibel T (2012) Interactions of fibroblasts with different morphologies made of an engineered spider silk protein. Adv Eng Mater 14(3):B67–B75. doi:10.1002/adem.201180072

Lee KS, Kim BY, Je YH, Woo SD, Sohn HD, Jin BR (2007) Molecular cloning and expression of the C-terminus of spider flagelliform silk protein from Araneus ventricosus. J Biosci 32(4):705–712. doi:10.1007/s12038-007-0070-8

Lewis RV, Hinman M, Kothakota S, Fournier MJ (1996) Expression and purification of a spider silk protein: a new strategy for producing repetitive proteins. Protein Expres Purif 7(4):400–406. doi:10.1006/prep.1996.0060

Lin Z, Huang W, Zhang J, Fan JS, Yang D (2009) Solution structure of eggcase silk protein and its implications for silk fiber formation. Proc Natl Acad Sci USA 106(22):8906–8911. doi:10.1073/pnas.0813255106

Madani F, Lindberg S, Langel U, Futaki S, Graslund A (2011) Mechanisms of cellular uptake of cell-penetrating peptides. J Biophys 2011:10 p. doi:10.1155/2011/414729

Mello CM, Soares JW, Arcidiacono S, Butlers MM (2004) Acid extraction and purification of recombinant spider silk proteins. Biomacromolecules 5(5):1849–1852. doi:10.1021/Bm049815g

Menassa R, Hong Z, Karatzas CN, Lazaris A, Richman A, Brandle J (2004) Spider dragline silk proteins in transgenic tobacco leaves: accumulation and field production. Plant Biotechnol J 2(5):431–438. doi:10.1111/j.1467-7652.2004.00087.x

Mieszawska AJ, Nadkarni LD, Perry CC, Kaplan DL (2010) Nanoscale control of silica particle formation via silk-silica fusion proteins for bone regeneration. Chem Mater 22(20):5780–5785. doi:10.1021/Cm101940u

Morgan AW, Roskov KE, Lin-Gibson S, Kaplan DL, Becker ML, Simon CG Jr (2008) Characterization and optimization of RGD-containing silk blends to support osteoblastic differentiation. Biomaterials 29(16):2556–2563. doi:10.1016/j.biomaterials.2008.02.007

Motriuk-Smith D, Smith A, Hayashi CY, Lewis RV (2005) Analysis of the conserved N-terminal domains in major ampullate spider silk proteins. Biomacromolecules 6(6):3152–3159. doi:10.1021/bm050472b

Numata K, Kaplan DL (2010) Silk-based gene carriers with cell membrane destabilizing peptides. Biomacromolecules 11(11):3189–3195. doi:10.1021/Bm101055m

Numata K, Subramanian B, Currie HA, Kaplan DL (2009) Bioengineered silk protein-based gene delivery systems. Biomaterials 30(29):5775–5784. doi:10.1016/j.biomaterials.2009.06.028

Numata K, Reagan MR, Goldstein RH, Rosenblatt M, Kaplan DL (2011) Spider silk-based gene carriers for tumor cell-specific delivery. Bioconjug Chem 22(8):1605–1610. doi:10.1021/bc200170u

Numata K, Mieszawska-Czajkowska AJ, Kvenvold LA, Kaplan DL (2012) Silk-based nanocomplexes with tumor-homing peptides for tumor-specific gene delivery. Macromol Biosci 12(1):75–82. doi:10.1002/mabi.201100274

Partis MD, Griffiths DG, Roberts GC, Beechey RB (1983) Cross-linking of protein by omega-maleimido alkanoyl N-hydroxysuccinimido esters. J Protein Chem 2(3):263–277. doi:10.1007/BF01025358

Prince JT, Mcgrath KP, Digirolamo CM, Kaplan DL (1995) Construction, cloning, and expression of synthetic genes encoding spider dragline silk. Biochemistry 34(34):10879–10885. doi:10.1021/bi00034a022

Rammensee S, Slotta U, Scheibel T, Bausch AR (2008) Assembly mechanism of recombinant spider silk proteins. Proc Natl Acad Sci USA 105(18):6590–6595. doi:10.1073/pnas.0709246105

Rathore O, Sogah DY (2001) Self-assembly of beta-sheets into nanostructures by poly(alanine) segments incorporated in multiblock copolymers inspired by spider silk. J Am Chem Soc 123(22):5231–5239. doi:10.1021/Ja004030d

Rising A, Hjalm G, Engstrom W, Johansson J (2006) N-terminal nonrepetitive domain common to dragline, flagelliform, and cylindriform spider silk proteins. Biomacromolecules 7(11):3120–3124. doi:10.1021/bm060693x

Schacht K, Scheibel T (2011) Controlled hydrogel formation of a recombinant spider silk protein. Biomacromolecules 12(7):2488–2495. doi:10.1021/Bm200154k

Scheibel T (2004) Spider silks: recombinant synthesis, assembly, spinning, and engineering of synthetic proteins. Microb Cell Fact 3(1):14. doi:10.1186/1475-2859-3-14

Schmidt M, Romer L, Strehle M, Scheibel T (2007) Conquering isoleucine auxotrophy of *Escherichia coli* BLR(DE3) to recombinantly produce spider silk proteins in minimal media. Biotechnol Lett 29(11):1741–1744. doi:10.1007/s10529-007-9461-z

Seidel A, Liivak O, Jelinski LW (1998) Artificial spinning of spider silk. Macromolecules 31(19):6733–6736. doi:10.1021/ma9808880

Seidel A, Liivak O, Calve S, Adaska J, Ji GD, Yang ZT, Grubb D, Zax DB, Jelinski LW (2000) Regenerated spider silk: processing, properties, and structure. Macromolecules 33(3):775–780. doi:10.1021/ma990893j

Sletten EM, Bertozzi CR (2009) Bioorthogonal chemistry: fishing for selectivity in a sea of functionality. Angew Chem Int Ed 48(38):6974–6998. doi:10.1002/anie.200900942

Spiess K, Wohlrab S, Scheibel T (2010) Structural characterization and functionalization of engineered spider silk films. Soft Matter 6(17):4168–4174. doi:10.1039/B927267d

Sponner A, Unger E, Grosse F, Weisshart K (2004) Conserved C-termini of spidroins are secreted by the major ampullate glands and retained in the silk thread. Biomacromolecules 5(3):840–845. doi:10.1021/bm034378b

Sponner A, Vater W, Rommerskirch W, Vollrath F, Unger E, Grosse F, Weisshart K (2005) The conserved C-termini contribute to the properties of spider silk fibroins. Biochem Biophys Res Commun 338(2):897–902. doi:10.1016/j.bbrc.2005.10.048

Stark M, Grip S, Rising A, Hedhammar M, Engstrom W, Hjalm G, Johansson J (2007) Macroscopic fibers self-assembled from recombinant miniature spider silk proteins. Biomacromolecules 8(5):1695–1701. doi:10.1021/Bm070049y

Szela S, Avtges P, Valluzzi R, Winkler S, Wilson D, Kirschner D, Kaplan DL (2000) Reduction-oxidation control of beta-sheet assembly in genetically engineered silk. Biomacromolecules 1(4):534–542. doi:10.1021/Bm0055697

Teulé F, Aubé C, Ellison M, Abbott A (2003) Biomimetic manufacturing of customised novel fibre proteins for specialised applications. AUTEX Res J 3(4):160–165

Teulé F, Furin WA, Cooper AR, Duncan JR, Lewis RV (2007) Modifications of spider silk sequences in an attempt to control the mechanical properties of the synthetic fibers. J Mater Sci 42(21):8974–8985. doi:10.1007/s10853-007-1642-6

Teulé F, Cooper AR, Furin WA, Bittencourt D, Rech EL, Brooks A, Lewis RV (2009) A protocol for the production of recombinant spider silk-like proteins for artificial fiber spinning. Nat Protoc 4(3):341–355. doi:10.1038/nprot.2008.250

Teulé F, Addison B, Cooper AR, Ayon J, Henning RW, Benmore CJ, Holland GP, Yarger JL, Lewis RV (2011) Combining flagelliform and dragline spider silk motifs to produce tunable synthetic biopolymer fibers. Biopolymers 97(6):418–431. doi:10.1002/bip.21724

Thordarson P, Le Droumaguet B, Velonia K (2006) Well-defined protein-polymer conjugates-synthesis and potential applications. Appl Microbiol Biotechnol 73(2):243–254. doi:10.1007/s00253-006-0574-4

Valluzzi R, Szela S, Avtges P, Kirschner D, Kaplan D (1999) Methionine redox controlled crystallization of biosynthetic silk spidroin. J Phys Chem B 103(51):11382–11392. doi:10.1021/jp991363s

Vendrely C, Scheibel T (2007) Biotechnological production of spider-silk proteins enables new applications. Macromol Biosci 7(4):401–409. doi:10.1002/mabi.200600255

Vendrely C, Ackerschott C, Roemer L, Scheibel T (2008) Molecular design of performance proteins with repetitive sequences: recombinant flagelliform spider silk as basis for biomaterials. Methods Mol Biol 474:3–14. doi:10.1007/978-1-59745-480-3_1

Vollrath F, Madsen B, Shao ZZ (2001) The effect of spinning conditions on the mechanics of a spider's dragline silk. Proc R Soc Lond B 268(1483):2339–2346. doi:10.1098/rspb.2001.1590

Wang M, Yu JH, Kaplan DL, Rutledge GC (2006) Production of submicron diameter silk fibers under benign processing conditions by two-fluid electrospinning. Macromolecules 39(3):1102–1107. doi:10.1021/Ma0517749

Wen HX, Lan XQ, Zhang YS, Zhao TF, Wang YJ, Kajiura Z, Nakagaki M (2010) Transgenic silkworms (*Bombyx mori*) produce recombinant spider dragline silk in cocoons. Mol Biol Rep 37(4):1815–1821. doi:10.1007/s11033-009-9615-2

Widmaier DM, Voigt CA (2010) Quantification of the physiochemical constraints on the export of spider silk proteins by Salmonella type III secretion. Microb Cell Fact 9:78. doi:10.1186/1475-2859-9-78

Widmaier DM, Tullman-Ercek D, Mirsky EA, Hill R, Govindarajan S, Minshull J, Voigt CA (2009) Engineering the *Salmonella* type III secretion system to export spider silk monomers. Mol Syst Biol 5:309. doi:10.1038/msb.2009.62

Winkler S, Szela S, Avtges P, Valluzzi R, Kirschner DA, Kaplan D (1999) Designing recombinant spider silk proteins to control assembly. Int J Biol Macromol 24(2–3):265–270. doi:10.1016/S0141-8130(98)00088-9

Winkler S, Wilson D, Kaplan DL (2000) Controlling beta-sheet assembly in genetically engineered silk by enzymatic phosphorylation/dephosphorylation. Biochemistry 39(41):12739–12746. doi:10.1021/Bi001335w

Winningham MJ, Sogah DY (1997) A modular approach to polymer architecture control via catenation of prefabricated biomolecular segments: polymers containing parallel beta-sheets templated by a phenoxathiin-based reverse turn mimic. Macromolecules 30(4):862–876. doi:10.1021/ma960804s

Wohlrab S, Mueller S, Schmidt A, Neubauer S, Kessler H, Leal-Egana A, Scheibel T (2012) Cell adhesion and proliferation on RGD-modified recombinant spider silk proteins. Biomaterials 33(28):6650–6659. doi:10.1016/j.biomaterials.2012.05.069

Wong Po Foo C, Patwardhan SV, Belton DJ, Kitchel B, Anastasiades D, Huang J, Naik RR, Perry CC, Kaplan DL (2006) Novel nanocomposites from spider silk-silica fusion (chimeric) proteins. Proc Natl Acad Sci USA 103(25):9428–9433. doi:10.1073/pnas.0601096103

Xia XX, Ki CS, Park YH, Kaplan DL, Lee SY (2010) Native-sized recombinant spider silk protein produced in metabolically engineered *Escherichia coli* results in a strong fiber. Proc Natl Acad Sci USA 107(32):14059–14063. doi:10.1073/pnas.1003366107

Xu M, Lewis RV (1990) Structure of a protein superfiber – spider dragline silk. Proc Natl Acad Sci USA 87(18):7120–7124. doi:10.1073/pnas.87.18.7120

Xu HT, Fan BL, Yu SY, Huang YH, Zhao ZH, Lian ZX, Dai YP, Wang LL, Liu ZL, Fei J, Li N (2007) Construct synthetic gene encoding artificial spider dragline silk protein and its expression in milk of transgenic mice. Anim Biotechnol 18(1):1–12. doi:10.1080/10495390601091024

Yang JJ, Barr LA, Fahnestock SR, Liu ZB (2005) High yield recombinant silk-like protein production in transgenic plants through protein targeting. Transgenic Res 14(3):313–324. doi:10.1007/s11248-005-0272-5

Zarkoob S, Eby RK, Reneker DH, Hudson SD, Ertley D, Adams WW (2004) Structure and morphology of electrospun silk nanofibers. Polymer 45(11):3973–3977. doi:10.1016/j.polymer.2003.10.102

Zhou CZ, Confalonieri F, Jacquet M, Perasso R, Li ZG, Janin J (2001) Silk fibroin: structural implications of a remarkable amino acid sequence. Proteins: Struct Funct Genet 44(2):119–122. doi:10.1002/prot.1078

Zhou CC, Leng BX, Yao JR, Qian J, Chen X, Zhou P, Knight DP, Shao ZZ (2006) Synthesis and characterization of multiblock copolymers based on spider dragline silk proteins. Biomacromolecules 7(8):2415–2419. doi:10.1021/Bm060199t

Zorko M, Langel U (2005) Cell-penetrating peptides: mechanism and kinetics of cargo delivery. Adv Drug Deliv Rev 57(4):529–545. doi:10.1016/j.addr.2004.10.010