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Engineered spidroin-derived high-performance fibers for diverse applications

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ABSTRACT

Spider silks are well known for their exceptional mechanical properties that are tougher than Kevlar and steel. However, the restricted production amounts from their native sources limit applications of spider silks. Over the decades, there have been significant interests in fabricating man-made silk fibers with comparable performance to natural silks, inspiring many efforts both for biosynthesizing recombinant spider silk proteins (spidroins) in amenable heterologous hosts and biomimetic spinning of artificial spider silks. These strategies provide promising routes to produce high-performance and functionally optimized fibers with diverse applications. Herein, we summarize the hosts that have been applied to produce recombinant spidroins. In addition, the fabrication and mechanical properties of recombinant spidroin fibers and their composite fibers are also introduced. Furthermore, we demonstrate the applications of recombinant spidroin-based fibers. Finally, facing the challenges in biosynthesis, scalable production, and hierarchical assembly of high-performance recombinant spidroins, we give a summary and perspective on future development.

KEYWORDS

spider silk, recombinant spidroin, heterologous expression, fiber fabrication, mechanical property

1 Introduction

Natural polymers have received considerable attention due to their sustainability. Spidroins, as the major and key protein components of spider silks, have received special attention among the many natural polymer materials [1]. Since the 1990s, the works related to recombinant spider proteins have been published. In recent years, the scope of recombinant spider silk field has expanded drastically, along with rapid development of synthetic and chemical biology. Spider silks are spun from specialized glands including the pyriform, flagelliform, aciniform, tubuliform, aggregate, major, and minor ampullate glands [2]. The spider can produce up to seven different varieties of silks, with dragline silk, also known as major ampullate silk or the spider lifeline, exhibiting the most remarkable strength and elasticity [3]. The dragline silk comprises several proteins and the primary ones are major ampullate spidroin 1 (MaSp1) and MaSp2 [4]. These spidroins comprise three domains, with the long repetitive central domain flanked by nonrepetitive amino- and carboxy-termini [5]. The central domain is characterized by the alternate arrangement of polyalanine (Poly-Ala) motif and glycine/proline-rich motif. The polyalanine motif enables the formation of hydrophobic crystalline β-sheet structures, whereas the hydrophilic glycine/proline-rich motif serves as the link between β-sheets and is responsible for the extensibility of silk fibers [6]. The characteristic modular sequences of MaSps lead to anti-parallel β-sheets nanocrystals in spider silks, which can further organize into hierarchical architectures at the nanometer or micrometer scales [7]. Notably, the two-terminal domains of MaSps are also required for hierarchical assembly [8]. Such multi-scale hierarchical structures are responsible for the enviable mechanical properties of spider silks [9].

Despite the superior mechanical properties of spidroins, it is difficult to obtain large amounts of spidroins from spider silks due to the low natural production as well as the cannibalism of spiders which are unfeasible for farming. Fortunately, with the development of genetic engineering and synthetic biology, recombinant spidroins with tunable sequences and chain length can be overexpressed through various hosts [10]. In addition, a variety of artificial spinning methods, including wet spinning [11, 12], microfluidic spinning [13], electrostatic spinning, and bioinspired spinning processes have been applied to produce tough protein fibers from recombinant spidroins [14]. By optimizing the spinning process, it is feasible to improve the performance of fibers in terms of controllable morphology, well-defined structure, and uniform thickness. Due to their excellent biocompatibility and biodegradability, recombinant spidroin fibers

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can be used for a wide range of applications, including tissue engineering, wound healing, drug delivery, etc. [15–17].

In this context, we summarize the various host cells used to express recombinant spidroins, especially bacterial hosts. Then the spinning methods and mechanical properties of recombinant spidroin fibers are discussed. Furthermore, the applications of recombinant spidroin fibers are also introduced, especially in biomedical fields. Finally, future challenges for the development of recombinant spidroin fibers have been prospected.

2 Production of recombinant spidroins in heterologous expression systems

There have been extensive efforts to produce biomimetic fibers by artificially spinning recombinant spidroins. To optimize the chain length, solubility, and final yield of recombinant spidroins, many attempts have been made to engineer expression hosts (Fig. 1 and Table 1), including bacteria, yeast, transgenic plants, animals, etc.

2.1 Bacterial expression systems

2.1.1 *Escherichia coli*

E. coli is one of the most commonly used expression hosts for recombinant spidroin production due to conventional genetic engineering and low manufacturing costs [18, 19]. It is very attractive to produce large-size recombinant spidroins, as the protein fibers derive their superior mechanical performance partially from their high molecular weights. However, alanyl- and glycyl-tRNA pools are often depleted in wild-type *E. coli* hosts as a result of the spidroin core domain's highly repetitive sequences [20]. The tRNA deficiency may lead to premature translation termination, resulting in the inability to express high-molecular weight spidroins. Cao et al. produced recombinant spidroins with a 110 kDa molecular weight. The code of glycyl/alanyl-tRNA was introduced into *E. coli* to provide more tRNA for protein translation. The yield of recombinant spidroins expressed in the flask after Ni chromatographic column purification and lyophilization was 150 mg/L [21]. Moreover, Xia et al. developed an efficient method to improve the glycol-tRNA pool, which increases the recombinant spidroins yield by about to 35-fold [22]. By using acetic acid and ammonium sulphate precipitation, the 96-mer protein with a molecular weight of 284.9 kDa was purified. The final yield was 1.2 g/L, and the purity was nearly 90%. This simple, non-chromatographic technique eliminates any potential truncation of the proteins during the purification procedure. Subsequently, to produce larger spidroin protein, Xia and colleagues demonstrated high-level synthesis of recombinant spidroins of 201.6 kDa by modifying the induction temperature [23]. The plasmid can be maintained by low-temperature induction at 16 °C. In a 3 L fed-batch bioreactor, a high output titer of around 3.6 g/L was attained. For producing high-molecular-weight protein, split inteins (SIs) may be the best option [24]. SIs are continuous polypeptides that catalyze protein splicing while only leaving a few residues at the ligation site. Bowen et al. chose SIs-based ligations to generate 556 kDa molecular weight recombinant spidroins [25]. The yield was about 2 g/L in the fermenter, and the *in vitro* ligation efficiency was 62%.

As shown in Fig. 1, natural spider silk proteins employed polyalanine sequences to form β-sheets conformation and make contributions to high tensile strength. Although the residues such as valine (Val) and isoleucine (Ile) are more β-prone when compared with alanine, due to their side chains that can mediate stronger interactions in β-sheets, polyvaline (poly-Val) or polyisoleucine (poly-Ile) might be too hydrophobic to pass through the translocon in the endoplasmic reticulum (ER) membrane [26]. This is the possible reason for why spiders do not use poly-Val or poly-Ile. Therefore, when poly-Val or poly-Ile sequences were introduced in recombinant spidroins, prokaryotic hosts would be a better choice because the target protein's translation and accumulation occur in the cytoplasm [27, 28]. Rising and colleagues designed 15 spidroin variants that contained other amino acids (Val, cysteine (Cys), Ile, and phenylalanine (Phe)) to replace Ala in the poly-Ala blocks of the repetitive region [29]. Six of the protein variants can be produced in a high yield (> 100 mg/L in the flask). Another manufacturing method developed by the team allowed more than 20 g/L of protein yield

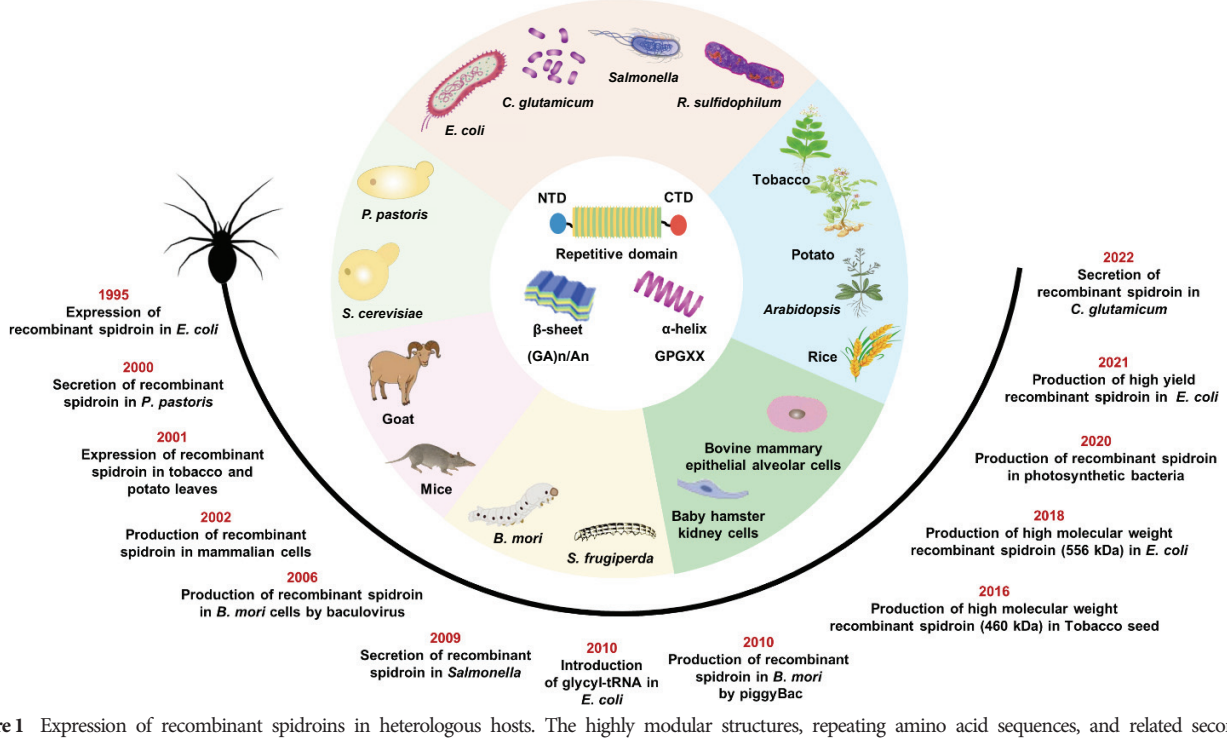

Figure 1 Expression of recombinant spidroins in heterologous hosts. The highly modular structures, repeating amino acid sequences, and related secondary structures of spidroins are shown in the inner panel. The outer panel shows the milestones in exploring the heterologous expression systems of recombinant spidroin.

in the fed-batch culture and 14.5 g/L of the target protein after purification [30].

2.1.2 Other bacteria

Recombinant spidroins contain highly repetitive and hydrophobic sequences, which often lead to expression as insoluble inclusion bodies in *E. coli* [31]. For subsequent protein purification, inclusion bodies are often dissolved under extremely harsh conditions and then dialyzed to remove the denaturant [32]. During the dialysis process, there is a risk of low protein recovery and accidental precipitation, resulting in increased purification costs. Therefore, compared with intracellular expression, secretion of recombinant spidroins in the culture medium provides several benefits, including excellent product stability, solubility, simplified downstream processing, and increased biological activity [33]. However, *E. coli* does not naturally secrete high amounts of protein. Thus, other bacterial expression systems have been explored.

Through both the inner and outer membranes, the type III secretion system (T3SS) transports protein from the cytoplasm to the outside medium. Widmaier et al. designed and produced spidroin variants in *Salmonella*, where T3SS is used to export synthetic spidroins from the bacteria cytoplasm [34]. It showed that 14% of expressed protein can be exported at a secretion rate of 1.8 mg/(L·h). To effectively secrete recombinant spidroins, *Corynebacterium glutamicum* was initially exploited by Xia and colleagues [35]. By optimizing the signal peptide, knocking out the gene encoding recombinase, increasing the tRNA pool, and changing the permeability of cell wall, the resulting MaSpI16 aqueous solution had a recovery yield of 84.9%, a purity of around 93.0%, and a protein content of about 100 mg/mL. Furthermore, the study by Foong et al. illustrated the synthesis of recombinant spidroins in *Rhodovulum sulfidophilum* under photoautotrophic or photoheterotrophic conditions [36]. Despite low yield, it provides a green and sustainable approach for heterologous spidroin expression.

2.2 Yeast expression system

Yeast host is capable of producing larger recombinant spidroins without premature translation termination, and secreting recombinant proteins into the media, thus simplifying purification steps [37]. Furthermore, yeast host like *Pichia pastoris* can utilize alternative carbon sources and be well amplified to industrial-scale fermentation.

Sidoruk et al. optimized the fermentation process of a *Saccharomyces cerevisiae* strain that produces recombinant spidroins, and the production of the target protein rose by almost 60% [38]. Meanwhile, under the control of the methanol-inducible AOX1 promoter, recombinant spidroins were produced at about 1 g/L in shake flask cultures in *P. pastoris*, as reported by Fahnestock et al. [39]. Unfortunately, the protein is not secreted in these studies. To improve the secretion efficiency, Ronnie et al. evaluated the effects of pH, temperature, and induction time on the secretion of recombinant spidroin by *P. pastoris* [40]. Although the secretion efficiency and protein yield can be optimized by modulating the above conditions, the target protein is easy to degrade in the supernatant. Thus, it remains challenging to improve the secretion efficiency and simultaneously reduce the degradation of target protein in yeast expression system.

2.3 Mammalian cells and insect cells expression systems

Compared with other systems, the mammalian cell expression system benefits the correct folding of proteins, so the protein products are closer to their natural counterparts. By adopting bovine mammary epithelial alveolar cells immortalized with big T (MAC-T) and baby hamster kidney (BHK) cells, Lazaris et al. attempted to imitate the process of spidroin production, and the yield of recombinant spidroins ranged from 25 to 50 mg/L [41]. Furthermore, 110 and 140 kDa soluble spidroins were found in the culture medium, indicating the capability of protein secretion.

Strategies employed for the production of recombinant spidroin have also included insect cells. The most significant advantage is that insects are evolutionarily closer to spiders [42]. For example, the *Spodoptera frugiperda* cell line Sf9 is used to generate recombinant spidroin, providing a new method for recombinant spidroin production [43–45].

2.4 Transgenic plants and animals expression systems

2.4.1 Transgenic plants

Transgenic tobacco and potato plants employed for producing recombinant spidroins were initially described by Scheller et al. [46]. The endoplasmic reticulum of tobacco and potato leaves and potato tubers, respectively contains at least 2% of the total soluble protein. One kilogram of tobacco leaf can provide 80 mg of pure recombinant spidroins after extraction [47]. Subsequently, the production of recombinant spidroin from tobacco leaf and tobacco seed was also reported, but the low yield in tobacco is still the biggest issue [48–51]. However, tobacco systems attract great attention since they can synthesize larger recombinant proteins than other systems.

Arabidopsis leaves and seeds are also employed to produce recombinant spidroins. In terms of quality and yield, seed-specific production outperformed leaf-based one [52]. Yang et al. used different signal peptides to make recombinant spidroins accumulate in specific cellular chambers. Soluble protein accumulation in the endoplasmic reticulum can reach 18% [53]. However, for rice and alfalfa expression systems, almost no specific yield has been reported. This might be attributed to gene silence, which is a common problem, especially for spidroins with highly repetitive sequences [10, 42, 54].

2.4.2 Transgenic animals

Unlike spiders, *Bombyx mori* can be grown extensively at low cost and thus produce vast amounts of fiber. Therefore, it is feasible to replace the silkworm gene with the spider gene to produce spider silks effectively. To express recombinant spidroins in *B. mori*, Miao et al. first developed a unique *B. mori* nuclear polyhedrosis virus (BmNPV) baculovirus expression system [55]. This study developed a speedy small-scale culture separation process and realized an expression level of around 13 g/mL. This team adopted this technology in another study, the expression level was about 45 μg/mL in larvae haemolymph [56]. To further increase the production level, Zhang et al. expressed spidroins in BmN cells and silkworm larvae by Bac-to-Bac/BmNPV baculovirus expression elements [57]. The recombinant spidroins expressed in the BmN cell probably accounted for 5% of the total protein or 6 mg in a silkworm larva. Recently, newly developed genome editing tools, such as piggyBac, transcription activator-like effector nucleases, and CRISPR/Cas9, have been successfully applied to insert the spidroin gene into the silkworm chromosome, which may open new capabilities for the large-scale production of high-performance protein fibers [58–62].

Other transgenic animals such as transgenic mice and sheep are also adopted. Recombinant spidroins were found in their milk and embryos, but the yield was too low. Nevertheless, transgenic

Table 1 Recombinant spidroins expressed in different heterologous hosts[a]

Hosts	Origin spider	Protein homolog	Size (kDa)	Maximum yield (mg/L)	Purification method	References
E. coli	*N. clavipes*	MaSp2	110	150	b	[21]
E. coli	*N. clavipes*	MaSp1	100.7–284.9	1200	a	[22]
E. coli	*N. clavipes*	MaSp2	28.3–256.5	3600	—	[23]
E. coli	*N. clavipes*	MaSp1	285 and 556	2000	a + b	[25]
E. coli	*Euprosthenops australis* and *Araneus ventricosus*	MaSp1 and MiSp	33	243	b	[29]
E. coli	*E. australis* and *A. ventricosus*	MaSp1 and MiSp	33	1450	b	[30]
Salmonella	*A. diadematus*	ADF1, ADF2, and ADF3	25–56	14	—	[34]
C. glutamicum	*Trichonephila clavipes*	MaSp1	42.2 and 168.9	567.9	a	[35]
R. sulfidophilum	*N. clavipes*	MaSp1	7.9–20.9	63.48	b	[36]
S. cerevisiae	*N. clavipes*	MaSp1	94	450	b	[38]
P. pastoris	*N. clavipes*	MaSp1	96.6	663	a	[39]
P. pastoris	*N. clavipes*	MaSp1	32	300	b	[40]
MAC-T and BHK	*N. clavipes* and *A. ventricosus*	ADF3, MaSp1, and MaSp2	60–140	50	a	[41]
S. frugiperda	*A. diadematus*	ADF4	60	50	b	[44]
S. frugiperda	*A. ventricosus*	FlSp	28–61	—	—	[45]
Tobacco leaf	*N. clavipes*	MaSp1	94.2	80 mg/kg	a	[47]
Tobacco leaf	*N. clavipes*	MaSp1	104	66.6 mg/kg	a	[48]
Tobacco leaf	*N. clavipes*	FlSp	72 and > 250	36 mg/kg	b	[49]
Tobacco seed	*N. clavipes*	FlSp	72 and > 460	190 mg/kg	—	[50]
Arabidopsis leaves	*N. clavipes*	MaSp1	64	18% of total proteins	b	[53]
B. mori	*N. clavipes*	FlSp	37	13 μg/mL	b	[55]
B. mori	*N. clavipes*	FlSp	37	45 μg/mL	b	[56]
B. mori	*N. clavipes*	MaSp1	70	6 mg per larvae	b	[57]
B. mori	*N. clavipes*	MaSp1	83	—	—	[58]
B. mori	*N. clavipes*	MaSp2 and FlSp	78–106	5% of composite fiber protein	—	[59]
B. mori	*A. ventricosus*	Like ADF3	~ 100	0.37%–0.61% of composite fiber protein	—	[66]
B. mori	*N. clavipes*	MaSp1	67	35.2% of composite fiber protein	—	[61]
Transgenic mice	*N. clavipes*	MaSp1 and MaSp2	40–55	11.7	—	[63]
Transgenic goats	*N. clavipes*	MaSp1 and MaSp2	65	—	—	[64]

[a] a represents precipitation recovery (salt precipitation or thermal precipitation), b is chromatography purification, and — indicates not reported.

animal systems address the concerns about endotoxin pollution and have great potential in the medical field [63–65].

3 Mechanical properties of recombinant spidroin fibers

3.1 Fibers spun from entire recombinant spidroins

The natural spider silk proteins are stored at high concentrations in the spinning gland. During the spinning process, the spinning dope passes through the tapered spinning tube, and the C-terminal domain (CTD) partially unfolds under the action of shear force to expose the hydrophobic surface, thus inducing the formation of the β-sheet structure [67]. Furthermore, the charged amino-terminal domains undergo pH-dependent dimerization, promoting the formation of N-terminal antiparallel dimers [68]. In addition, the kosmotropic ions such as phosphate ions also contribute to structure formation and protein aggregation [69]. All these factors promote protein assembly into preliminary fibers. Subsequently, hydrogen bonding between protein molecules increases under the influence of dehydration at the end of the spinning tube, which promotes the crystallization of protein molecules, transforming the nanofibrils into solid filaments [70]. With the further study of spiders, researchers have simulated the spinning process of spider silk to prepare high-performance fibers by wet spinning, microfluidic spinning, etc. [71]. Specifically, the pre-dissolved proteins are extruded into a coagulation bath and then solidified by the double diffusion effect between solvents to form fibers during wet spinning [72]. Microfluidic spinning produces microfibers with different sizes and morphologies through specific microchannels such as single channel, double channel, "core–shell" flow channel, etc. [73]. The mechanical properties of the recombinant spidroin fibers are shown in Table 2.

It is well known that the internal hierarchical structure of protein fiber affects its mechanical performance. Thomas and colleagues devised a biomimetic way to prepare fibers by wet spinning, to induce structural assembly in spidroin [74]. After

being dissolved in guanidine thiocyanate, the recombinant spidroins were dialyzed against sodium phosphate buffer. The C-terminal domain begins to partially refold as a result of the phosphate ions' addition, which triggers the beginning of micelle assembly in spinning dopes. This leads to the formation of extensive networks between protein molecules, which is necessary for the special mechanical properties of fibers. After 600% stretching, N1L(AQ)$_{12}$NR3 fibers exhibited a toughness of 189 ± 33 MJ/m^3, which was comparable to that of natural spider silk. This team then co-expressed recombinant eADF4 sequence and eADF3 sequence on the same plasmid and exploited the CTD domain to self-assemble into heterodimeric fibers with an excellent tensile strength of up to 834 ± 34 MPa (Fig. 2(a)) [75]. Fan et al. found that tubuliform spidroin 2 (TuSp2), the minor component of TuSp, played a key role in promoting the correct conformation of TuSp assembly [76]. The spun recombinant spidroin fibers based on the synergic action of the main components TuSp1 and TuSp2 have significantly higher tensile strength (760 ± 49 MPa) and Young's modulus (15 ± 1 GPa) than their native counterparts (Fig. 2(b)). Xu et al. prepared recombinant aciniform spidroin (AcSp) fibers and discovered that the CTD derived from AcSp1 can induce better folding of protein structure than others, thus leading to higher mechanical properties (Fig. 2(c)) [77].

Moreover, the mechanical characteristics of recombinant spidroin fibers are impacted by the protein molecular weight, as higher molecular weight leads to fewer defects in fibers. Xia et al. developed robust fibers with native-sized recombinant spidroins by wet spinning [22]. The tensile strength and extensibility of the 96-mer fibers were 508 ± 108 MPa and 15% ± 5%, respectively. Remarkably, Young's modulus was 21 ± 4 GPa, which is twice as high as the dragline silk (11–14 GPa). Meanwhile, Zhang and colleagues produced fibers made of a 556 kDa recombinant spidroin using wet spinning. The fibers outperform most natural spider silks in terms of tensile strength (1030 ± 110 MPa) and Young's modulus (13.7 ± 3.0 GPa) (Fig. 2(d)) [25]. Lin et al. prepared mechanically strong fibers with a 378 kDa recombinant TuSp that is slightly larger than natural ones. The mechanical performances (tensile strength and Young's modulus of 308 ± 57 MPa and 9.3 ± 3 GPa) are 30% and 50% greater than those of natural TuSp fibers, respectively [78]. Other studies on recombinant spidroins fibers derived from subordinate spinning glands such as flagelliform spidroin (FlSp), aggregate spidroin (AgSp), and AcSp, also confirm that the mechanical properties increase with protein molecular weight [79–81].

In addition, the fiber properties can also be improved by optimizing the spinning process. Xia and colleagues developed a device to manufacture shear forces by imitating the shearing gradients in microfluidics spinning [82]. The spinning dope was extruded into the ethanol coagulation bath. The extensibility and tensile strength of post-spin drawn fibers were up to 30% and 420 MPa, respectively. By adjusting the pH value of the aqueous coagulation bath and optimizing the concentration of spinning dope, Rising and colleagues produced kilometer-long fiber using wet spinning [83].

3.2 Recombinant spidroins-based composite fibers

Compared with entirely spidroin fibers, composite fibers with multiple components are expected to perform higher mechanical properties and stability [84–87]. By cross-linking with titanium dioxide (TiO_2) and formaldehyde (FA), Zhu et al. produced recombinant pyriform spidroin (PySp) composite fibers with a toughness up to 249 ± 22 MJ/m^3, exceeding most natural dragline silks (Fig. 2(e)) [88]. This result shows that the introduction of covalent bonds helps to improve the mechanical properties of fibers, which is conducive to achieving the goal of preparing high-performance fibers with low-molecular-weight recombinant spidroins.

According to the study by Xu et al., the mechanical properties of spidroin/fibroin composite fiber produced by silkworms are significantly improved [61]. Similarly, Kuwana et al. produced composite fiber composed of fibroin and dragline protein in cocoon silk, which has greater toughness (116.1 MJ/m^3) even higher than synthetic rubber (100 MJ/m^3) and the spider dragline

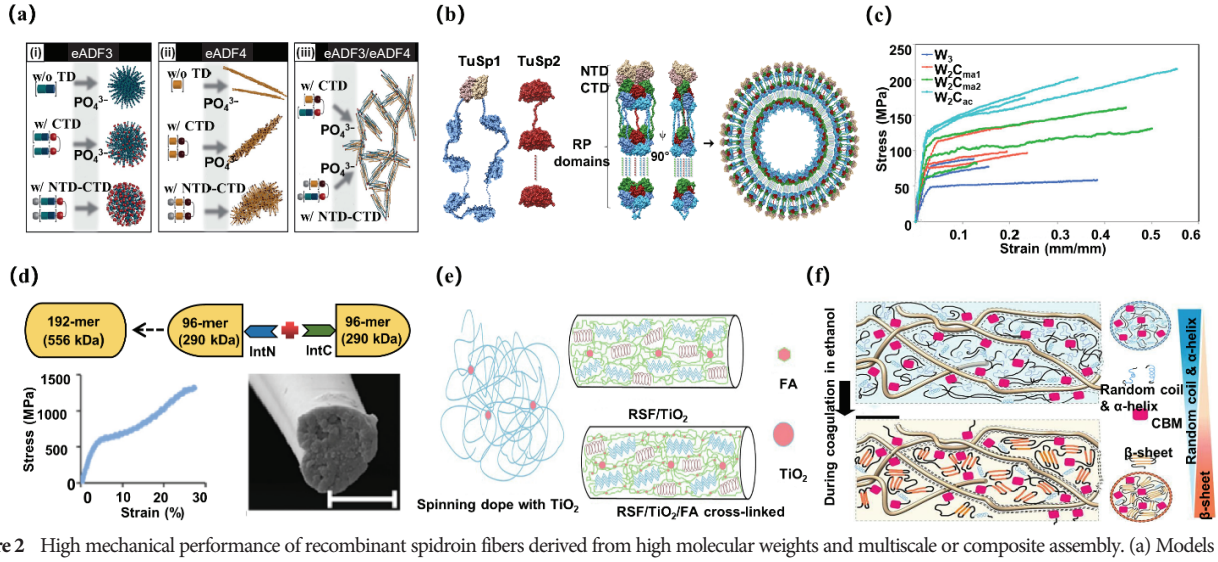

Figure 2 High mechanical performance of recombinant spidroin fibers derived from high molecular weights and multiscale or composite assembly. (a) Models of self-assembly of homo- and hetero-dimeric recombinant spidroins comprising eADF3-CTD/eADF4-CTD. Reproduced with permission from Ref. [75], © Saric, M. et al. 2021. (b) Structural models of TuSp1/TuSp2 complexes and micelle formation. Reproduced with permission from Ref. [76], © Fan, T. T. et al. 2021. (c) Stress–strain curves of three recombinant spidroin fibers. Comparison of amino acid compositions of W repeat unit from *Argiope trifasciata* AcSp1 and CTDs from *E. australis* MaSp1 (C_{ma1}), *A. trifasciata* MaSp2 (C_{ma2}), and AcSp1 (C_{ac}). Reproduced with permission from Ref. [77], © American Chemical Society 2017. (d) SIs-mediated covalent ligation of 96-mer spidroin to yield a 192-mer, 556 kDa product. The ligated product was purified and spun into fibers for mechanical testing. Scale bar: 5 μm. Reproduced with permission from Ref. [25], © American Chemical Society 2018. (e) Schematic diagrams of TiO_2 and FA cross-linking with protein. Reproduced with permission from Ref. [88], © Elsevier B.V. and Société Française de Biochimie et Biologie Moléculaire (SFBBM) 2020. (f) Schematic diagram of the change of CBM-ADF3-CBM conformation from the coacervate (blue at the top) to coagulation and drying (orange at the bottom). Reproduced with permission from Ref. [91], © Mohammadi, P. et al. 2019.

(*Nephila clavipes*, 111 MJ/m^3) [66]. Zhang et al. manufactured composite fibers that are as strong as dragline by incorporating spidroin genes into the silkworm genome [60]. Tang et al. designed two composite fibroin fibers containing two spidroin genes (AgSp gene and PySp gene), respectively [89]. The two genes both significantly enhanced the mechanical properties of composite fibers.

Mittal et al. combined cellulose nanofibrils (CNFs) with two types of recombinant spidroins and fabricated fibers using microfluidic technology [90]. The materials exhibit outstanding Young's modulus (~ 55 GPa), toughness (~ 55 MJ/m^3), and tensile strength (~ 1015 MPa). The composite fiber made by Mohammadi et al. contains proteins composed of three parts, the recombinant spidroin (*Araneus diadematus* fibroin 3 (ADF3)) sequence in the center and the cellulose-binding domain (CBM) sequence at both ends [91]. Remarkably, the composite fibers exhibit a high Young's modulus of 35 ± 6.3 GPa (Fig. 2(f)).

Table 2 Overview of mechanical properties of recombinant spidroin fibers[a]

Protein	Strength (MPa)	Toughness (MJ/m^3)	Modulus (GPa)	Extensibility (%)	Diameter (μm)	References
MaSp	370 ± 59	189 ± 33	4 ± 1	110 ± 25	27 ± 10	[74]
MaSp	834 ± 34	143 ± 6	5 ± 0.4	32 ± 1	27 ± 1	[75]
MaSp	508 ± 108	—	21 ± 4	15 ± 5	—	[22]
MaSp	1030 ± 110	114 ± 51	13.7 ± 3	18 ± 6	5.7 ± 1.3	[25]
MaSp	286.2 ± 137.7	37.7 ± 28.8	8.4 ± 4.3	18.3 ± 12.8	8.7	[82]
MaSp	288.7 ± 20.9	100.9 ± 13.2	3.78 ± 0.34	47.1 ± 3.7	34.1 ± 0.7	[92]
MaSp	131.63 ± 31.87	145.63 ± 42.18	3.5 ± 0.95	160.44 ± 37	4.16 ± 0.78	[29]
MaSp	162 ± 8	45 ± 7	6 ± 0.8	37 ± 5	12 ± 2	[83]
TuSp	308 ± 57	—	9.3 ± 3	~ 10	6–14	[78]
TuSp	760 ± 49	37 ± 5	15 ± 1	8–10	—	[76]
AcSp	175.1 ± 29	41.8 ± 24.1	5.5 ± 0.5	29 ± 13	2.1 ± 0.3	[77]
FlSp	253.65 ± 69.02	23.76 ± 16	9.61 ± 2.6	10.89 ± 6.27	3.02 ± 1	[79]
AgSp	37.6 ± 4.6	1.0 ± 0.2	1.1 ± 0.2	4.5 ± 0.8	29.2 ± 4.3	[80]
AcSp	117 ± 22	105 ± 13	5 ± 1	113 ± 15	14 ± 2	[81]
Composite fibers						
PySp/TiO_2/FA	167 ± 14	249 ± 22	6.3 ± 0.7	197 ± 20	8.9 ± 0.8	[88]
Spidroin/fibroin	338.4 ± 87	77.2 ± 29.5	5.49 ± 1.18	31.1 ± 4.5	20.6 ± 1.3	[59]
Spidroin/fibroin	1256.6 ± 280.8	246.8 ± 95.1	12.9 ± 3.2	0.3 ± 0.1	6.7 ± 0.6	[60]
Spidroin/fibroin	371.5 ± 27.5	84.8 ± 14.4	8.9 ± 1.3	32.2 ± 4.6	66.9 ± 8.4	[61]
Spidroin/fibroin	591.7 ± 35	116.1 ± 9.5	14.66 ± 1.06	27.5 ± 1.4	—	[66]
Spidroin/fibroin	344.34 ± 123.33	55.72 ± 32.84	7.31 ± 1.94	25–30	—	[89]
Spidroin/fibroin	337.57 ± 69.43	35.21 ± 15.16	7.21 ± 1.72	15–20	—	[89]
Spidroin/CNF	830 ± 24	~ 37	52.8 ± 0.7	~ 6	—	[90]
CBM-ADF3-CBM	478 ± 65	31.82 ± 3.2	25 ± 2.5	8–10	—	[91]

[a] — indicates not reported.

4 Applications of recombinant spidroin-based fibers

4.1 Biomedical applications

Spidroin fibers have been widely used in biomedical fields, such as drug delivery carriers, tissue engineering scaffolds, and antimicrobial and implantable devices, due to their excellent mechanical properties and good biocompatibility [93, 94]. Although silk proteins have also been applied in biomedical fields and they are more easily accessible than spidroins, silks exhibit inferior mechanical properties compared with spider silks. This difference may be attributed to the more hydrophobic β-sheets crystalline region formed by polyalanine in spidroin, which leads to tighter intermolecular actions and thereby higher material strength [95]. Therefore, spidroins are more advantageous than silk proteins in biomedical fields such as tissue scaffolds which need high mechanical requirement.

Chen et al. created spidroin nanofiber membrane that can control the release of insulin-like growth factors and promote wound healing [96]. Nanofibrous membrane composed of sodium hydrogen sulfide (NaHS) and recombinant spidroins was explored by Lian et al. [97]. Such material possesses good cytocompatibility and hemocompatibility and contributes to stable and sustained release of H_2S, thus considerably improving wound regeneration efficiency (Fig. 3(a)).

Safonova et al. made electrospun scaffolds composed of silkworm protein and recombinant spidroin containing RGD sequence (arginine-glycine-aspartic acid) that can enhance the molecule's cytocompatibility and sticky characteristics [98]. The scaffolds sped up skin healing by 19 days in comparison with negative control and were fully biodegradable when the skin healed (Fig. 3(b)). Furthermore, composite scaffolds containing recombinant spidroin have been proven to promote cell growth [99]. Zhou et al. combined recombinant spidroin ($NTW_{1-4}CT$) with poly(L-lactic-co-ε-caprolactone) (PLCL) to produce nanofibrous scaffolds [100]. The hydrophobic scaffold surface became hydrophilic as the number of repeat sequences (W) of

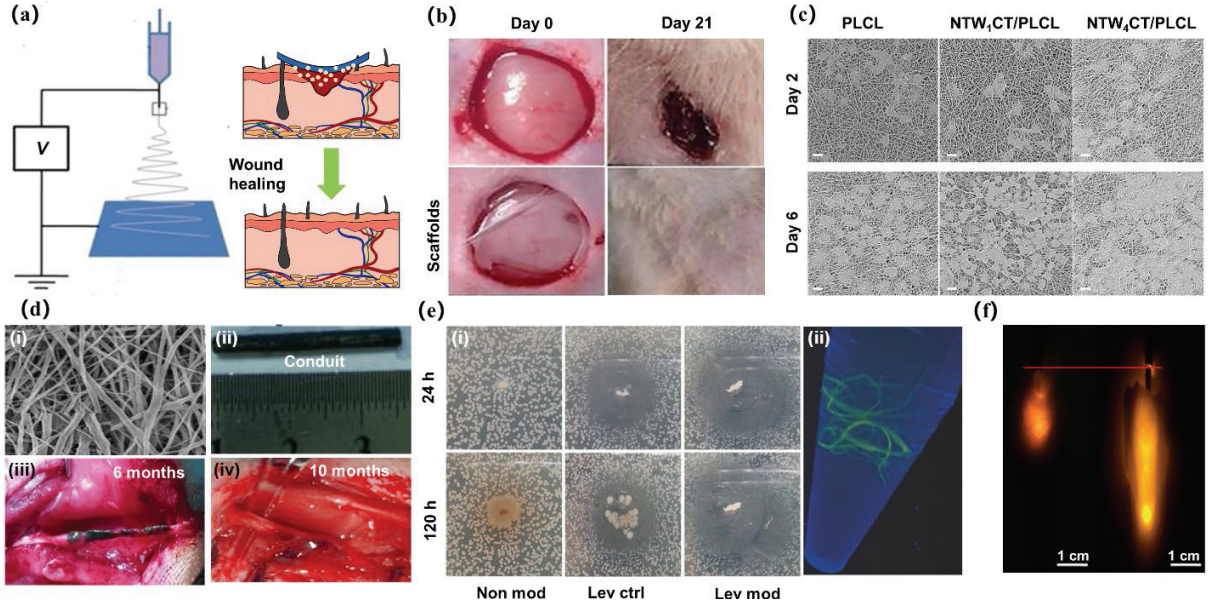

Figure 3 Recombinant spidroin fibers for biomedical applications. (a) Electrospun nanofibers membranes to promote wound healing through sustained drug release. (b) Images of wounds from experimental days 0 and 21. Compared with no scaffolds above, the bottom scaffolds healed after 21 days. Reproduced with permission from Ref. [98], © Safonova, L. et al. 2021. (c) Scanning electron microscopy (SEM) images of cells cultivated for 2 and 6 days on blend spidroin scaffolds and pure PLCL scaffolds. Scale bar: 20 μm. Reproduced with permission from Ref. [100], © Elsevier B.V. 2020. (d) (i) SEM image of fibrous scaffolds. (ii) Macroscopic view of the electrospun conduit. (iii) The segment of regenerated nerve-like tissue at 6 months. (iv) The scaffold successfully bridging the sciatic nerve gap within 10 months. Reproduced with permission from Ref. [105], © Elsevier B.V. 2015. (e) (i) The inhibition zone of three materials against *E. coli*, containing nonfunctionalized (Non mod) fibers, levofloxacin control (Lev ctrl) fibers, and levofloxacin-functionalized (Lev mod) fibers. (ii) Successful coupling of recombinant spidroin fibers with fluorophores. Reproduced with permission from Ref. [107], © WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 2016. (f) Light penetration lengths with (left) and without (right) recombinant spidroin fibers inserted into the muscle. Reproduced with permission from Ref. [108], © American Chemical Society 2017.

spidroin increased. Compared with pure PLCL scaffolds, composite scaffolds provide superior support for cell proliferation (Fig. 3(c)). Meanwhile, Yang et al. mineralized CaP on the surface of recombinant spidroin fibers, which is conducive to the attachment and growth of human mesenchymal stem cells derived from bone marrow [101, 102]. Furthermore, recombinant spidroins have been spun into vascular scaffolds, which showed excellent biocompatibility and non-immunogenicity. The scaffold can serve as a good candidate for small diameter vascular tissue engineering [103].

Recombinant spidroin scaffolds also show great applicability in the high-tech field of nervous system repair. Yu and colleagues synthesized three-dimensional (3D) multi-porous recombinant spidroins/poly(L-lactic acid) (PLLA) fibrous scaffold, which directed the extension of the axon through aligned hierarchical structure [104]. Then, using co-axial electrospraying and electrospinning, this team created composite fibrous conduit and successfully closed a 2 cm sciatic nerve gap in adult mice within 10 months (Fig. 3(d)) [105]. Hansson et al. synthesized a functionalized spidroin carrying a peptide derived from vitronectin (VN-NT2RepCT), which successfully promoted nerve growth and axon extension [106].

In addition, recombinant spidroin fiber also plays an important role in antimicrobial and implantable devices. Harvey et al. produced fluorescent and antibacterial fibers by linking azidohomoalanine into recombinant spidroin and then coupling the fluorophores and levofloxacin on the fiber via the click chemistry method [107]. After 5 days, the levofloxacin "click" functionalized fiber still maintains antibacterial activity (Fig. 3(e)). Xia and colleagues discovered that the recombinant spidroin fibers had a smoother surface, a better refractive index, and a lower optical loss compared with regenerated fibroin fiber [108]. Taking advantage of these features, recombinant spidroin fibers successfully transmitted light to deep tissue (3 cm) when placed into muscle, which is promising for applications in the fields of *in situ* imaging, continuous monitoring, etc. (Fig. 3(f)).

4.2 Other applications

The rich side-chain groups of recombinant spidroins endow the protein with a great possibility of chemical modification and fiber functionalization. Furthermore, natural spider silks possess unique characteristics, such as shape-memory behavior, humidity response, etc. All these characteristics potentially enable broader applications of recombinant spidroin materials.

Thomas and colleagues prepared Janus fibers with complex dual-functional structures from two recombinant spidroins with opposite charges. The maleimide-functionalized gold nanoparticles (AuNPs) are coupled on one side of the fiber, endowing the fiber with good conductivity (Fig. 4(a)) [109]. Moreover, by centrifugal electrospinning, this team produced spidroin nanofiber nonwoven. The lowest filtration efficiency of this nonwoven was 94% for particles as small as 0.2 μm, comparable to commercial filters (Fig. 4(b)) [110]. Cheng et al. modified CuS nanoparticles and AuNPs on the surface of recombinant spidroin fibers through coordination and electrostatic interaction [111]. The functional fibers showed great promise for usage as photothermal and ultraviolet (UV)-resistant materials (Fig. 4(c)). Yuan et al. synthesized spidroin-based super uranyl-binding protein (SSUP) fiber by combining the spidroin gene and super uranyl-binding protein gene [112]. The hydrogel-like structure of the SSUP fibers gave plenty of hydrophilic intermolecular spaces for uranyl ions to enter, generating fibers with extremely high uranium extraction ability in seawater of 12.33 mg/g and ultra-rapid equilibration time of 3.5 days (Fig. 4(d)). Super-contractile features of draglines served as inspiration for Venkatesan et al. to fabricate functional recombinant spidroin fibers [113]. The fibers demonstrated humidity-triggered shape memory behavior at 75% relative humidity (RH), with a good shape fixity of 82.1% ± 2.1% and a total recovery ratio of 98.5% ±

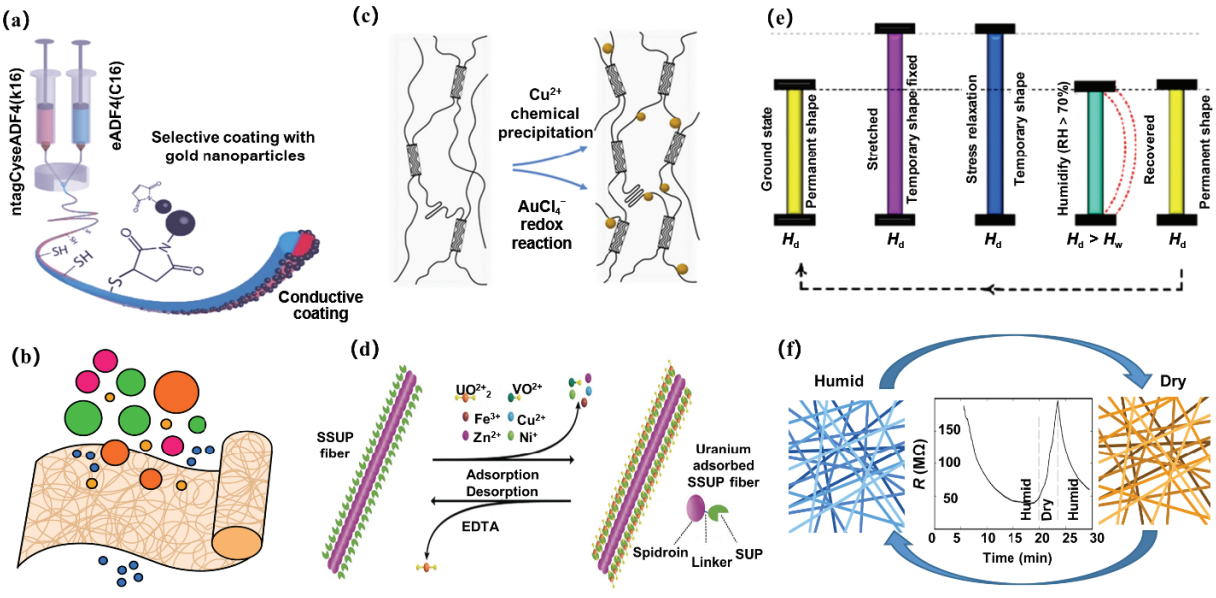

Figure 4 Recombinant spidroin fibers with potential applications in conducting electricity, air purification, photothermal conversion, metal extraction, and humidity sensing. (a) Schematic diagram of the preparation of Janus fiber covalently bound with AuNPs. Reproduced with permission from Ref. [109], © Lang, G. et al. 2022. (b) Nonwoven fabric prepared from recombinant spidroin is used for filtration. (c) Functionalization of fibers by the integration of NPs via chemical precipitation or redox reaction. Reproduced with permission from Ref. [111], © American Chemical Society 2022. (d) The mechanism for uranium adsorption and desorption by spidroin-based uranyl sequestration protein fiber. Reproduced with permission from Ref. [112], © Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim 2019. (e) Schematic diagram of humidity-triggered shape memory behavior of spidroin fibers, where H_d is dry state and H_w is wet state. Reproduced with permission from Ref. [113], © The Royal Society of Chemistry and the Chinese Chemical Society 2019. (f) Cyclic resistance of the nanocomposite at RH level of 90%. Reproduced with permission from Ref. [114], © Shehata, N. et al. 2018.

0.4%. In addition, the fibers show a recovery tensile strength of 18.5 ± 0.5 MPa at 90% RH (Fig. 4(e)). Piezoelectric nano-fiber was produced by Shehata et al. via electrospinning [114]. The applied voltage can be detected sensitively through mechanical deformation. Additionally, the fiber produced measurable resistance at RH values above 70% and exhibited cyclic changes according to the periodic dry/wet conditions (Fig. 4(f)).

5 Conclusions and prospects

Spider silk has been a major focus of biomimetic materials due to its extraordinary mechanical and biological properties. With the development of biosynthetic technology and material science, recombinant spidroin fibers have been extensively investigated. Herein, a variety of host systems for expressing recombinant spidroins are summarized. These strategies help to solve the problem of difficult-to-obtain spidroins and greatly promote the applications of recombinant spidroins. Meanwhile, the commonly used spinning process and mechanical properties of entirely spidroin fibers or composite fibers are discussed. The mechanical properties of recombinant spidroin fibers can be optimized even comparable to native counterparts. In addition, the versatility of recombinant spidroin fibers and composite fibers allows for various applications.

Despite the great progress in the study of recombinant spidroin fibers, there are still some challenges that need to be addressed. Generally, production yield is an important indicator of industrial applications. However, the current yields of recombinant spidroins are still severely limited. Furthermore, the current secretion efficiency is low, resulting in high costs of downstream purification. In addition, there remain disparities between the comprehensive mechanical properties of artificially spun fibers and natural spider silk. Therefore, it is necessary to explore more effective hosts, to increase the production of existing host cells, and to optimize purification methods. On the other hand, deep understanding of the structure and silk-forming conditions of natural spider silk and deeply simulating the natural spinning process are promising strategies to optimize the mechanical properties of the fibers. In conclusion, further efforts are still needed to realize industrial-scale production of high-performance recombinant spidroin fibers.

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