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Review

Structure, production and application of spider silks

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ABSTRACT

Spider silk plays a pivotal role in the diverse physiological activities of spiders, with its protein components exhibiting remarkable mechanical properties and biocompatibility. Spider silk proteins exhibit a high degree of repetitiveness, primarily constructed through the recurring arrangement of amino acid motifs, including $(A)_n$, $(GA)_n$, $(GGX)_n$, and $(GPGXX)_n$ sequences. These repetitive sequences endow spider silk with different material properties. Recombinant spider silk proteins are produced through heterologous expression systems, and then spun into nanofibers using artificial spinning technology. These fibers have broad potential applications in the biomedical field, such as tissue engineering scaffolds, drug delivery carriers, sutures, and other biomaterials. However, enhancing the yield and performance of recombinant spider silk proteins, while facilitating large-scale production, continues to pose a significant challenge in the current landscape.

1. Introduction

For centuries, spider silk, a sustainable biomaterial, has been utilized across multiple fields. The intricate protein composition and molecular architecture of natural spider silk confer upon it remarkable mechanical properties, including high strength, exceptional extensibility, high tensile stress tolerance, and low density. The prepared spider silk protein fibers have great potential for applications in fields such as medicine, military, and tissue engineering [1–5]. For example, the tensile strength of dragline silk, reported to be 1.1 GPa, surpasses that of silkworm silk (0.6 GPa) and stands as the strongest non-mineralized biological material known to date [3,6]. Meanwhile, its high tensile strength and toughness have led to the development of spider silk as a bulletproof material [7]. Due to its high biocompatibility, spider silk fibers have been developed into medical materials such as sutures and hemostatic stickers [8,9]. Despite the territorial and cannibalistic nature of spiders, the production of spider silk remains limited and challenging to acquire [10]. Therefore, researchers are committed to exploring suitable host systems to obtain a large amount of recombinant spider silk protein [11], and then spinning it through different equipment and methods to prepare high-performance spider silk protein fibers [2,12].

This review summarizes the structural properties of spider silk, showcasing its unique advantages. At the same time, we list methods for producing recombinant spider silk proteins and delve into their promising applications in the biomedical field, emphasizing the potential for future advancements in recombinant spider silk from a "structure-production-application" trinity perspective.

2. Literature search and selection criteria

We conducted preliminary literature searches using PubMed (https://pubmed.ncbi.nlm.nih.gov/), Web of Science (https://webofscience.clarivate.cn/wos/alldb/basic-search) and Wanfang Database (http://wff.a.xue66.net/) as data sources. Aiming to cover a wide range of publications based on spider silk proteins, we conducted preliminary literature searches using the following keywords: spider silk protein, structure, recombinant expression, biological medicine, and tissue engineering applications. From the last literature search conducted for this review in December 2024, back to the year 2000, and with the language restriction of the literature being English, we ultimately retrieved 6577 publications. For the initial articles obtained, we further defined inclusion and exclusion criteria: Inclusion criteria included (1) original articles; and (2) articles highly relevant to the topic of this review. Exclusion criteria included (1) conference abstracts, proceedings, editorial materials, or other non-article documents; (2) preprints, corrections, or retracted publications; (3) articles with low relevance to the topic; (4)

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articles overlapping in research content and conclusions with other articles; and (5) articles of poor quality in terms of structure, process, or writing. The final analysis included 202 publications, and the figures/images were designed using PowerPoint.

3. Types and properties of spider silk

After >400 million years of evolution, spiders have developed seven kinds of spider silk with different functions(Fig. 1) [13,14]. Spider silk not only participates in vital physiological activities such as hunting, mating, reproduction, and rapid movement, but also aids in early warning and escape, hence serving as crucial survival tools for spiders [15–18]. Despite the different origins and functions, their peptide chains share similar features in structure and composition, namely, flanking non-repetitive N-terminal (NT) and C-terminal (CT) domains on both sides, and a central core domain (Rep) [19,20]. Specifically, the non-repeating regions of the N-terminal (NT) and C-terminal (CT) sequences in spider silk proteins are approximately 130 bp in length and exhibit high conservation across diverse spider species. This conservation is crucial for the storage, dissolution, and self-assembly functions of spider silk proteins, particularly at high concentrations [14,21,22]. In contrast, Rep sequences have lower conservation and are composed mostly of repeating amino acid motifs. These include poly(alanine) ((A)n), glycine and alanine ((GA)n), an amino acid triad consisting of two glycine residues and a third variable amino acid (GGX)n, as well as the glycine-proline-glycine module ((GPGXX)n) [23]. Reps in different species are composed of repeated modules of varying lengths, endowing spider silk with unique material properties (Table 1).

The dragline silk produced by the main ampulla gland is currently one of the most extensively studied spider silk proteins, mainly composed of spidorin proteins, including two important isoforms: major ampulla spidorin 1 and 2 (MaSp1 and MaSp2) [24]. These two isoforms are rich in glycine GGX, GPGXX, GPGPX (X = Y, L or Q) motifs and $(A)_n$ (n = 4–12) motifs that help form specific secondary structures. The high tensile strength of dragline protein originates from its anti-parallel β-sheet structure, which is mainly formed by the extension of $(GA)_n$ and $(A)_n$ motifs in MaSp repeat sequences [25–28]. The GGX of MaSp1 forms a disordered 3_{10}-helix. In contrast, MaSp2, which is rich in GPGXX motifs, exists in the form of elastin-like type II β-turn conformations. Thus, drag fibers composed of MaSp2 exhibit several times the ductility and toughness compared to high-performance steel and Kevlar fibers [2,29–34]. Collin et al. identified overlapping groups of MaSp3 terminal regions from *Araneidae*, *Nephilidae*, and *Theridiidae* families using proteomics and genomic target capture techniques [35]. MaSp3 also has a typical $(A)_n$ motif and glycine-proline-glycine (GPG) motif. In addition, the content of serine and threonine increases, which is related to the special toughness of spider silk [36,37]. The MaSp4 and MaSp5 discovered in Darwin's bark spider belong to newly identified homologous proteins, and their core structural repeat domains are mainly composed of GGX motifs, as well as "VSVVSTTVS" and "GGLGGSG" sequence motifs, respectively [38,39]. These motifs may contribute to the resilience of spider silk, however, their detailed sequence information and specific mechanisms of action in spider silk production are still unclear and require further exploration [39].

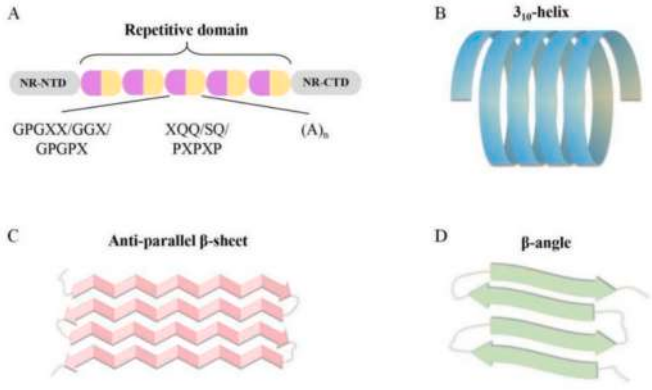

Fig. 1. Schematic structure of spider silk protein including (A) repetitive amino acid motifs and the corresponding secondary structures, such as (B) 3_{10}-helix, (C) anti-parallel β-sheet and (D) β-angle.

Minor ampullate silk is composed of two proteins, minor ampullate spiroin 1 and 2 (MiSp1 and MiSp2), both of which contain similar repeat sequences, mainly including repeat modules such as GX, GXX, GXXX, and $(A)_n$, which can promote the formation of 3_{10}-helices and anti-parallel β-folds [13,40–42]. Unlike MaSp, MiSp's A_n and $(GA)_n$ repeat motifs exhibit a tendency to form shorter β-fold structures. This characteristic leads to irreversible deformation of the ampulla gland filaments during stretching, ultimately resulting in slightly lower tensile strength but higher extensibility, as reported by Vienneau-Hathaway et al. and Lin et al. [34,43]. At the same time, when dragline silk is exposed to aqueous environments, a significant supercontraction phenomenon occurs, with its length reducing to approximately 50 % of its original size [44]. Similarly, MiSp fibers treated with water maintain a constant diameter while exhibiting a smaller degree of shrinkage compared to dragline silk fibers, demonstrating their potential value as protein delivery carriers [45].

Flagliform spider silk is mainly composed of flagelliform spider protein (Flag), and it serves as the capture silk of spiders [16,17,46]. When prey approaches the net, Flag wrap around the prey with their extremely high extensibility, making it difficult for the prey to escape [47]. Most Flag proteins are composed of glycine-rich GPGG $(X)_n$, which may be the reason for the formation of β-turn structures [48]. The continuous GGX motif provides Flag proteins with a large amount of β-helix structures, thereby giving flagellar filaments high elasticity and 200 % extensibilit [34,49]. In addition, unlike other spider silks, flagliform silk lacks an $(A)_n$ motif but has a highly conserved spacer motif whose main structural function remains unknown [48].

Aciniform spider silk exhibits remarkable high tensile strength and extensibility. It is predominantly utilized for entrapping prey and constructing protective structures known as oocysts [50]. Aciniform spider protein (AcSp) is the main component of the aciniform spider silk, absent of characteristic motifs such as GGX/GPGPX in the repetitive core region of other spider silk proteins [34]. Specifically, the AcSp1 protein isolated from *Argiope trifasciata* contains a higher percentage of other amino acids such as serine, leucine, and valine, thereby endowing aciniform spider silk with different mechanical properties [51,52]. In stark contrast to other AcSp proteins discovered thus far, AcSp2 lacks any significant similarity, and the unique TTX motif found within it may endow AcSp2 with completely different characteristics [50,53]. These unique compositional features of AcSp2 suggest potential novel mechanical and functional properties that warrant further investigation.

The tubuliform gland and ovary mature simultaneously, with the tubuliform silk protein (TuSp) secreted by the gland being responsible for both generating egg sacs and protecting spider eggs, playing a crucial role in reproduction. It exhibits extraordinary mechanical properties, particularly in terms of high stiffness compared to other spider silks [34,54]. The TuSp is rich in sulfur and contains long repetitive units of approximately 200 amino acid residues [55]. These repetitive units consist of (A)n, Sn, (SA)n, AX, and SQ motifs, which show very high intraspecific homogeneity and variable lengths between species, and can be arranged into β-sheet structures with large interlayer spacings [56]. These structures are prone to deformation and twisting, resulting in lower tensile strength of the tubuliform gland silk [12,34,57,58].

Pyiform spider silk, which spiders utilize to interconnect various silk fibers or anchor silk to surfaces, is primarily composed of pyiform spidroin (PySp) [59]. The two terminal domains of PySp protein contain a range of four to five α-helices, which are thought to control the protein to form fibers or gels through self-assembly [60,61]. In the core repeat

Table 1
The function, core protein, and motif of each spider silk protein.

Silk gland	Type of fiber	Function	Core protein	Characteristic motif	References
Major ampullate gland	Major ampullate silk (Dragline silk)	Form the skeleton of the spider web, fall and escape	MaSp1, MaSp2, MaSp3, MaSp4, MaSp5	PolyGA or $(GA)_n$, polyA or $(A)_n$, GGX, GPGXX, GPGPX	Hou et al. [13], Kluge et al. [30], Malay et al. [24], Garb et al. [38]
Minor ampullate gland	Minor ampullate silk	Make the auxiliary spiral of the orb-web to stabilize the scaffold	MiSp1, MiSp2	$(GA)_n$, $(A)_n$, GGX, GX, GXX, GXXX	Hou et al. [13], Lin et al. [34], Yang et al. [41], Peng et al. [42]
Flagellate gland	Flagelliform silk	Catch the prey and mitigate the impact	Flag	$(GA)_n$, GGX, GPGG$(X)_n$	Römer and Scheibel [44], Lefèvre et al. [25], Tokareva et al. [26]
Aciniform gland	Aciniform silk	Wrap and secure prey	AcSp1, AcSp2	GGX, XQQ, GPGXX, GPGPX	Hou et al. [13], Peng et al. [42], Hayashi and Lewis [47], Ramezaniaghdam et al. [12]
Tubuliform gland	Tubuliform (cylindriform) silk	Protect spider eggs (only secreted by females)	CySp, TuSp1, ECP-1, ECP-2	$(A)_n$, $(S)_n$, $(SA)_n$, SQ, GX	Lin et al. [34], Peng et al. [42], Ramezaniaghdam et al. [12]
Piriform gland	Pyriform silk	Connect the web to the medium	PySp1, PySp2	$(PX)_n$, PXPXP, XQQ	Lin et al. [34], Ramezaniaghdam et al. [12]
Aggregate gland	Aggregate silk	Stick prey to prevent escape	AgSp1, AgSp2	GGX, GPGGX	Peng et al. [42], Ramezaniaghdam et al. [12]

region, both proline rich PXPXP motifs and QQ containing motifs can be observed. The pyriform silk is comprised of two spidroins, PySp1 and PySp2. All reported pyriform spidroins contain two unique motifs, namely the proline-rich (PX)n alternating region and the glutamine-rich (QQ) [61]. Among them, the (PX)n motif appears to form random coils, while the regions containing QQ form α-helical or β-sheet conformations, both of which contribute to the aggregation or self-assembly of PySp [12,62].

4. Expression and production of spider silk protein

Currently, two types of spider silk proteins, namely natural and recombinant proteins, have been the focus of extensive research and application. Natural spider silk proteins exist as highly concentrated (~50 %) solutions within the silk glands, where they self-assemble under specific conditions to form distinct layers of spider silk fibers. There are currently three main speculations about this process: (1) the liquid crystal theory that changes the spinning tube to affect the reorientation of spider silk protein chains and remove moisture, thereby forming natural layered fibers [24]. (2) The micelle hypothesis suggests that the flow and shear forces generated in the conduit can promote the deformation of MaSp's spherical structures, thereby forming discrete oligomeric structures (micelles) with hydrophobic elements inside and hydrophilic residues outside [63]. (3) Additionally, in the liquid-liquid phase separation method, the liquid-liquid phase separation of MaSp2 at neutral pH leads to the formation of silk protein droplets. Ultimately, as the pH decreases, the droplets become fibrous and form a hierarchical fiber structure [24,64,65]. These three perspectives delve into the intrinsic mechanisms of spider silk self-assembly from multiple dimensions such as structure or physiology. However, obtaining spider silk protein and fiber directly from spider webs, egg sacs, or glands results in low yields. Additionally, the process is often accompanied by impurities such as dust and prey debris, potentially posing a direct threat to the life of spiders, leading to their death [2,66]. To overcome the limitations of low yields and impurities associated with direct extraction from spider webs, egg sacs, or glands, researchers have turned to synthetic biology methods for the efficient production of recombinant spider silk proteins.

Genetic engineering technology has been applied to different hosts, such as bacteria [31,67–69], Yeast [1,70], Insects [71–78] or mammalian cell [79–83], and genetically modified plants (such as tobacco, potatoes, rice, or corn) [77,84–85] can express recombinant spider silk proteins.As early as 1995, Prince et al. successfully expressed the dragline silk protein gene from *Nephila clavipes* in *Escherichia coli* and validated its characterization and β-sheet structure [86]. However, this system suffers from low yield and is prone to gene deletions, transcription errors, and translation errors, all of which are attributed to premature termination of the synthesis process [67,87]. In contrast, the *Pichia pastoris* expression system has been demonstrated to produce stable genes, achieving high yields, reduced truncation, and enabling extracellular secretion, thus offering significant advantages [1,70]. Scheller et al. expressed high heat stable MaSp1 analogs in tobacco and potatoes, demonstrating that recombinant MaSp can be produced in plants [88–90]. In addition, the principle of protein targeting and post-translational protein fusion technology are also employed to solve the genetic and transcriptional instability problems caused by high repeatability of spider silk sequences, thereby increasing spider silk protein production [91–94]. The mammalian cell expression system is capable of promoting the correct folding and assembly of proteins. Specifically, bovine mammary epithelial alveolar cells, goat mammary cells, and hamster kidneys have all been successfully utilized to express spider silk protein genes, including MaSp1, MaSp2, and ADF-3 [79–83] (Fig. 2).

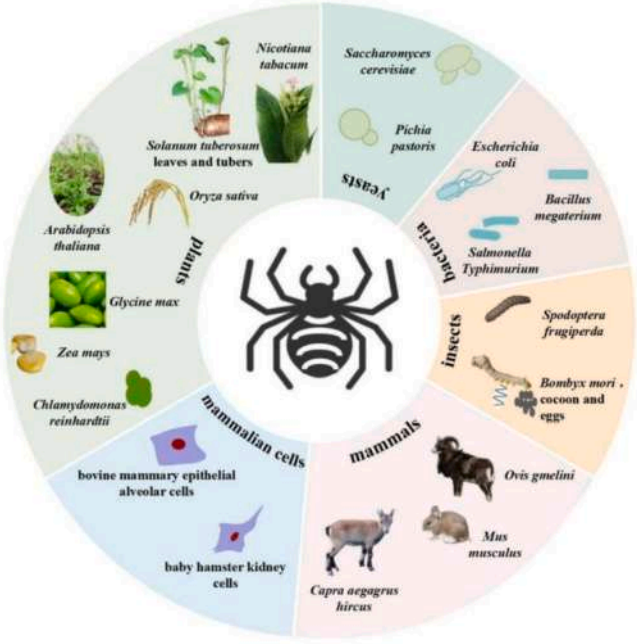

Fig. 2. Schematic diagram of heterologous expression system of recombinant spider silk protein [12].

5. Application of recombinant spider silk protein fibers

Biotechnology-generated recombinant spider silk proteins exhibit the capability to modulate their properties via both chemical and genetic modifications, as demonstrated by Aigner et al. [95]. For instance, incorporating functional peptides, notably the RGD sequence (consisting of arginine, glycine, and aspartic acid), significantly enhances the interaction between cells and silk proteins, regulating cell signaling, migration, differentiation, and cell movement [96,97]. Combining different amino acid sequences, such as replacing glutamic acid residues in the core domain repeat unit with lysine, can generate positively charged eADF4(C16) variants, improving cell adhesion [9,98]. Consequently, spider silk-based materials exhibit immense potential for applications in the burgeoning biomedical sector. Here, we will focus on the research status and application prospects of spider silk based materials from two aspects: tissue engineering and drug delivery carriers.

5.1. Tissue engineering

Tissue Engineering (TE) is a technique that combines cells with biocompatible, biodegradable, and absorbable biomaterials (scaffolds) and implants them into damaged tissues or organs of the body, in order to achieve the goal of repairing wounds and rebuilding function [99]. As a key component of TE, scaffolds are commonly used for functional reconstruction of defective tissues or damaged organs. This requires scaffold materials to have corresponding mechanical strength, be modifiable into different forms and structures, and provide sufficient space for material information exchange between tissues and cells [100]. In addition, the scaffolds should have good biocompatibility to reduce inflammation and cytotoxicity [101,102]. Currently, the extracellular matrix (ECM), which is secreted by tissues or organs, serves as a widely utilized natural scaffold material. ECM maintains a 'dynamic reciprocal' state with adhering cells and provides both biochemical and physical connections for newly formed cells and tissues [103–106]. Alginate [107,108], as well as natural polymers such as cellulose [109] and chitosan [110] have also been extensively studied for specific biological and mechanical functions. Additionally, synthetic polymers, including polylactic acid (PLA), polyurethane (PU), poly(lactide-*co*-glycolide) (PLGA), and polycaprolactones (PCL), exhibit excellent properties and degradation rates, and have been widely used in TE [111]. However, despite their advantages, both natural and synthetic polymers possess limitations that hinder their widespread clinical application. Natural polymers are expensive to produce and exhibit unstable mechanical properties. In contrast, synthetic polymers are less expensive to produce but often lack the biocompatibility necessary for tissue engineering applications. Therefore, extensive and in-depth research is still needed to optimize the synthesis process of polylactic acid-hydroxyl acetate copolymers (PLGA) as well as the precise control of their degradation rate [112]. Therefore, researchers have been dedicated to exploring a biomaterial that combines the advantages of natural and synthetic polymer materials over the past few decades.

5.1.1. Vascularization

Spider silk protein, renowned for its exceptional mechanical properties and biocompatibility, holds immense potential as an innovative biological scaffold material. Recombinant spider silk fibroin scaffolds have been shown to effectively induce endogenous angiogenesis via the arteriovenous (AV) loop mechanism, a process that facilitates blood vessel formation [113]. In addition, Sun et al. employed electrospinning technology to create spider silk protein scaffolds, subsequently implanting endothelial cells (ECs) and smooth muscle cells (SMCs) into their respective inner and outer layers, thereby constructing vascular grafts, which were then cultured in a dynamic bioreactor system. Dynamic cultivation of spider silk protein scaffolds results in denser cell growth and increased infiltration; it also maintains mechanical properties, including ultimate tensile stress (3.2 ± 0.33 MPa), Young's modulus (2.5 ± 1.7 MPa), and average rupture strength (1996 ± 168 mmHg), which are comparable to those of native blood vessels. This may be due to the fact that dynamic cultivation provides the necessary nutrients for cell growth through circulation and dilutes toxic cellular metabolites such as NH and lactate. After transplanting the graft into rats and culturing it for 9 weeks, it was difficult to distinguish between the graft and the rat's own artery, and no significant bleeding was observed at the anastomosis site. The number of macrophages and inflammatory cytokines also decreased with culture time [114]. The encouraging results presented herein underscore the potential of spider silk protein biomaterials in TE, paving the way for further research into optimizing their use in various tissue engineering applications. This type of biomaterial and bioreactor, which can passively transport body fluids, is beneficial for metabolism and enhances tissue regeneration. It also provides an optimized solution for directional electrospinning scaffold technology in tissue engineering [115].

5.1.2. Cardiac tissue engineering

The material made from recombinant spider silk protein has been conclusively proven to be suitable for cardiac tissue engineering. During the interaction between primary rat cells and recombinant spider silk protein eADF4 (κ 16) membrane, eADF4 (κ 16) membrane exhibits good adhesion properties to the most important cell types in cardiac tissue engineering (cardiomyocytes, which are the main muscle cells of the heart; endothelial cells, lining the interior surface of blood vessels; fibroblasts, producing collagen and other structural proteins; and smooth muscle cells, contributing to the contractility of vessels and organs) [66,116]. In addition, cardiomyocytes grown on eADF4 (κ 16) membrane have appropriate responses to extracellular stimuli and exhibit appropriate excitation propagation. These results indicate that eADF4 (κ 16) membrane is a promising new material for cardiac tissue engineering [117]. The eADF4 variant prepared by Esser et al. based on eADF4 (κ 16) also has high biocompatibility and does not cause non-specific immune responses [118]. On membranes made from eADF4 variants, human induced pluripotent stem cells (hiPSC) cardiomyocytes can effectively attach, diffuse, contract, and respond to drug therapy. However, selective cell behavior is observed on membranes made from different spider silk variants, underscoring the need for further investigation into the underlying mechanisms governing this phenomenon and the identification of suitable application scenarios tailored to each variant [119]. Future studies should focus on elucidating the molecular interactions between spider silk variants and various cardiac cell types, as well as evaluating the long-term functionality and stability of these membranes in preclinical models.

5.1.3. Bone regeneration

Bones consist of a blend of primarily inorganic materials, namely calcium phosphate, and organic materials, predominantly collagen. Many different materials and methods have been used in the development of bone tissue scaffolds [120]. Titanium metal [121,122], ceramics [123], polyetheretherketone [124], and other materials have been used to manufacture components in bone tissue, but these materials may cause issues such as body inflammation, metal sensitivity, and toxicity [120]. To mitigate these concerns, research has shifted towards the development of biodegradable composite biomaterials for bone tissue scaffolds. Therefore, developing a biodegradable composite biomaterial as a bone tissue scaffold has become a current research hotspot. Scaffolds based on poly (butylene terephthalate) (PBT) or poly (butyl terephthalate-*co*-poly (alkylene glycol) terephthalate) (PBTAT) have been shown to enhance bone growth through their favorable surface properties and biodegradability, making them suitable substrates for the in vitro attachment and proliferation of chondrocytes, mammalian skeletal muscle cells, and human mesenchymal stem cells [125,126]. On the film prepared by recombinant spider silk protein eADF4 (C16) and PBT or PBTAT composite polymer solution, the adhesion level of fibroblasts is higher than that of eADF4 (C16) membrane alone, which increases the

alkaline phosphatase activity of human mesenchymal stem cells cultured on the matrix, indicating that the composite material of eADF4 (C16) and PBT or PBTAT has certain potential in bone tissue engineering [120].

Building upon these findings, silk-based fusion proteins have been further explored for their potential in enhancing osteogenic differentiation. The hydroxyapatite binding domain, VTKHLNQISQSY (abbreviated as VTK), plays a pivotal role in inducing biomineralization. Dinjaski et al. (2017) fused the VTK domain to the N-terminus, C-terminus, or both ends of spider silk protein and discovered that when the VTK domain binds to both termini, the formation of crystalline hydroxyapatite significantly increases. Furthermore, all membrane forms of the resulting recombinant proteins supported the growth, proliferation, and differentiation of human mesenchymal stem cells (hMSCs) [127]. Analogously, silk-silica fusion proteins have been shown to support the growth and osteogenic differentiation of hMSCs, exhibiting an increase rate of osteogenic differentiation up to 85 % [128].

5.1.4. Wound healing and skin regeneration

In recent years, spider silk has also been explored for applications in skin regeneration and wound healing. Researchers have constructed epidermal skin using natural or recombinant spider silk proteins as a platform to support cell encapsulation and co-culture [129]. For example, natural spider silk has been shown to promote the release of inflammatory cytokines in full-thickness skin wounds in ovine. This was observed in experiments conducted in ovine, where single capillaries were seen to grow inward between spider silk fibers, forming new blood vessels, which play an important role in wound healing [130]. Primary human urethral epithelial cells (HUCs) can adhere, survive, and proliferate on spider silk without significantly altering their cellular characteristics, indicating that spider silk fibers are potential biomaterials for bladder reconstruction. Furthermore, studies have demonstrated that primary human urethral epithelial cells (HUCs) can adhere, survive, and proliferate on spider silk without significantly altering their cellular characteristics, suggesting spider silk fibers as potential biomaterials for bladder reconstruction [131].

Previous experiments have demonstrated that the Anthraea assama silk fibroin protein (AaSF) functionalized with recombinant spider silk fusion protein FN-4RepCT (FN-4RC) can enhance the adhesion, viability, and proliferation of various cell types, including dermal fibroblasts and endothelial cells, under in vitro conditions. This enhancement was achieved using freeze-dried microporous scaffolds [132]. When studying the efficacy of cell-free silk grafts in treating full-thickness wounds, Chouhan's team implanted the scaffold (coated with FN-4RC) in a rat burn model in vivo. After 14 days of treatment, the AaSF stent coated with FN-4RC promoted wound healing and showed a trend towards vascularization and epithelial reformation [133]. At the same time, the team also used two different types of recombinant spider silk proteins to coat AaSF nanofiber mats: FN-4RepCT (containing cell binding motifs derived from fibronectin) and Lac-4RepCT (containing cationic antimicrobial peptides derived from lactoferrin), and prepared them as wound dressings. Through the in vivo study of diabetes animal models, the results showed that the wounds with double coating (AaSF-FN-Lac) dressings healed fastest and completely within 12–14 days. The single coating (AaSF-FN and AaSF-Lac) dressings still had 15–18 % of the remaining wound area on the 12th day, significantly less than the uncoated (AaSF: 24 ± 2.09 %) dressings and the control groups (69 ± 6.45 % and 88 ± 6.39 %, respectively) [134]. The spider silk coating on silk fibroin protein enhances the healing effect by providing additional biological activity of fibronectin (FN) and lactoferrin (Lac). In conclusion, the spider silk coating on AaSF not only promotes wound healing but also enhances angiogenesis and epithelial formation, highlighting its great potential for skin repair applications [133,134].

5.1.5. Peripheral nerve repair

Peripheral nerve injuries (PNIs), which can significantly impact patients' quality of life, have long been a challenge in regenerative medicine. Despite advances in surgical techniques and physical therapies, effective methods to promote axonal regeneration and restore nerve function remain elusive [135,136]. The commonly used method currently is to use physical therapy measures such as surgery to facilitate direct repair of autologous nerve grafts. However, this method is limited to short-segment injuries, and factors such as the type of nerve, proximity to the damaged area, and age at the time of injury can all affect the regenerative ability of the injured nerve [137,138]. Another important treatment strategy is to implant biodegradable and biocompatible artificial neural conduits to help axons extend from the proximal end to the distal stump [9,139]. In nerve regeneration, Schwann cells (SC) guide axonal regeneration by secreting neurotrophic factors. In previous studies by Allmelling et al., it has been confirmed that spider silk fibers can promote SC adhesion and proliferation [140–142]. In subsequent experiments, researchers inserted homologous vein and spider silk vein filled grafts into the sciatic nerve space of rats. Histological analysis showed that although the walls of all catheters were penetrated by neovascularization, the sciatic nerve regeneration was good in the group treated with homologous veins or spider silk, with nerve fibers, nerve outer membranes, and nerve bundle sheaths at the distal end, exhibiting typical wave patterns of healthy nerves, while the control group transplanted with only homologous veins and matrix gel was filled with extensive void areas [143]. This study underscores the feasibility of spider silk fibers as promising guiding materials for peripheral nerve regeneration, not only as an ideal internal structure for nerve regeneration but also as a supportive matrix for axonal growth and SC migration [144,145].

On the other hand, Radtke et al. used decellularized vein grafts filled with spider silk fibers as guiding materials to bridge a 6.0 cm tibial nerve defect in adult sheep [146]. Four months post-surgery, the sheep implanted with spider silk fibers demonstrated the ability to walk in a coordinated and upright manner, utilizing both hind limbs effectively. During the subsequent 10-month observation period, histological analysis of the transverse sections of the transplanted autologous nerve and spider silk implants revealed that the axons were longitudinally arranged at the site of regenerative injury, followed by the formation of myelin sheath. The spider silk slowly degraded in the body without any signs of inflammation [146]. Kornfeld et al. detailed the axonal regeneration process at the site of implantation of spider silk nerve constructs in a 6.0 cm tibial nerve defect in adult sheep (*Ovis orientalis aries*). From the initial 20 days post-implantation, initial contact between axon structures and spider silk fibers was visibly observed. 90 days after implantation, nerve regeneration at the proximal end of the spider silk nerve structure was almost completely completed. This finding further demonstrates that spider silk nerve conduits exhibit comparable effects to autologous nerve grafts in facilitating nerve regeneration [147].

Previous experiments have fully demonstrated the important role of spider silk fiber conduits in nerve repair [140–143,146,147]. On this basis, Kornfeld and Radtke transplanted spider silk nerve conduits, inoculated with Schwann cells (SCs), into injury sites of varying lengths (4.0 cm, 10.0 cm, and 15.0 cm) in a rat model, to further investigate the repair efficacy of these conduits in addressing nerve injuries of differing severities. After 7 and 21 days of cultivation, live SCs were detected in catheters of different lengths, and on day 21, the arithmetic mean proliferation index of SCs reached 49.42 %. This indicates that spider silk nerve conduits provide a favorable environment for SC attachment, proliferation, and distribution at a distance of at least 15.0 cm in vitro, making them a highly suitable biomaterial for nerve reconstruction [148]. Semmler et al. [149] designed a new silk conduit, utilizing a tube wall composed of silkworm silk fibroin protein and filled with traction silk fibers from *Triconcephala edulis*. This composite material combines the processability of silk fibroin with the cell adhesion properties of traction silk, enhancing the regenerative potential of silk fiber nerve conduits and offering a promising autologous transplantation option for peripheral nerve injury rehabilitation [149] (Fig. 3).

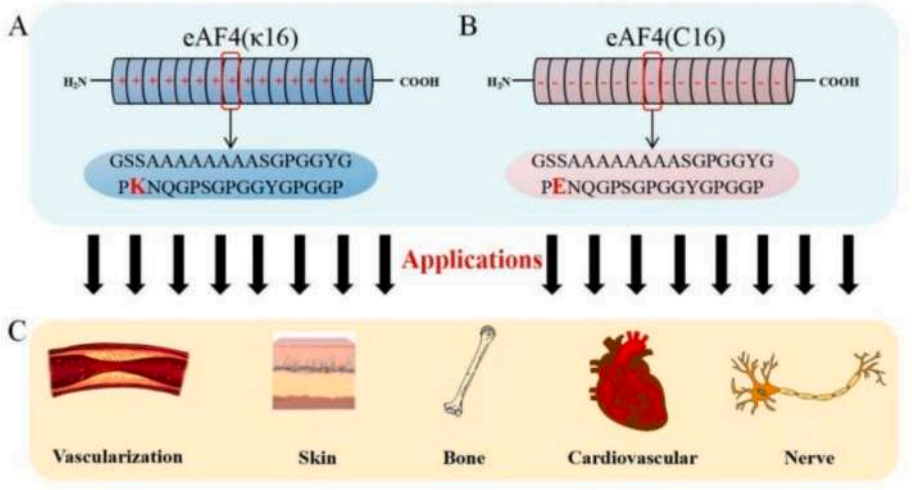

Fig. 3. The consensus sequence of the repetitive core domain of recombinant spider silk protein and its application in tissue engineering. (A) The recombinant engineered spider silk proteins eADF4(κ16) (positive net charge) and (B) eADF4(C16) (negative net charge) comprise 16 repeats of the respective modules. (C) The applications in tissue engineering [66,116].

5.2. Drug delivery

In recent years, silk fibroin, derived from both silkworms and spiders, has gradually emerged as a popular research focus for drug delivery carriers [150–152]. The Drug Delivery System (DDS) can not only target the release of drugs [153], but also minimize side effects and improve drug efficacy [154–156]. For example, a targeted delivery system based on silk fibroin nanoparticles (SFNP) can be used to load the cell growth inhibitor compound Gemcitabine (Gem) for the treatment of lung tumor induced mice [157]. Spider silk protein possesses various favorable attributes, including high biocompatibility, mechanical strength, and biodegradability [9,158–160]. However, its unique elasticity and slower degradation rate differ from those of silk [161]. And spider silk protein has good adjustability and is easy to chemically modify [153,162]. The genetic modification through the recombinant production process of spider silk protein has broad application prospects in cancer treatment and other fields [16,164].

The previously constructed recombinant engineered spider silk protein eADF4 (C16) can self-assemble into nanofibers in phosphate solution [165–167].Furthermore, it can form stable microspheres, enriched in beta sheets, as drug carriers in potassium phosphate solutions of high concentration (>400 mM) [150,158,168]. The mechanism of loading and releasing drugs is as follows: (1) Drug molecules are attracted to eADF4 (C16) particles via electrostatic interactions. (2) Once the surface of the particles becomes saturated, low molecular weight drugs begin to diffuse into the biopolymer matrix. Following loading and incubation in the release medium, the drug molecules are driven by concentration gradients towards the surface of the particles. Over time, the drug molecules gradually release, forming a stable release rate [158]. During this process, the efficiency of loading or releasing drugs onto eADF4 (C16) drug carriers is influenced by pH [158,169]. VThe efficiency of drug loading and release onto eADF4 (C16) drug carriers is influenced by various factors, including cross-linked proteins [167] and solvents [171,172]. For example, Herold et al. (2020) prepared a pH-sensitive carrier responsive system by coupling *para*-dimethylaminobenzaldehyde (DMAB) with hydrazine-modified eADF4 (C16) protein. With the acidification of the carrier environment, drugs can be slowly and continuously released from the corresponding carrier, maintaining a continuous supply of drugs [164]. The recombinant spider-eggcase-silk spheres designed through genetic engineering not only release drugs faster in acidic (pH = 4.5) environments, but also have a cumulative drug release of approximately 4.5 times that of pH 7.4 after 96 h. Furthermore, these spheres exhibit good control over drug loading in neutral PBS solutions, optimizing electrostatic interactions potentially suitable for lysosomal drug delivery [169]. In the past, spider silk films prepared from organic solvents such as hexafluoro-2-propanol required additional processing through phosphate ion or alcohol treatment to increase their β-folding content [173,174]. In addition to these advancements, novel film casting processes have been developed to streamline the fabrication process. The all aqueous film casting process developed by the Agostini team can effectively simplify this process and avoid damage from organic solvents to therapeutic protein drugs [175]. Diverse adapter designs facilitate the binding of recombinant spider silk protein carriers to specific enzymes or antigens [176]. Consequently, targeted drug delivery becomes achievable. These research results all indicate that the recombinant spider silk protein carrier drug delivery system has significant potential in improving therapeutic efficacy, enhancing drug resistance, and reducing adverse reactions. Continuing to optimize the design of carriers is expected to achieve more precise, efficient, and safe drug delivery in vaccine development, cancer treatment, and the treatment of various other diseases [98,177–179].

5.3. Other fields

Over the past thirty years, three-dimensional (3D) printing technology has achieved many significant breakthroughs in aerospace, food industry, and manufacturing sectors. At the same time, the potential applications of this technology in the field of biomedical engineering have gradually gained the attention of numerous researchers [180]. Collagen, silk fibroin, recombinant spider silk protein, and gelatin are protein polymers that can be combined with other synthetic polymers to prepare bio-inks for medical devices and tissue engineering [181–183]. The recombinant spider silk protein eADF 4 (C16) can self-assemble to form a hydrogel containing BALB/3T3 mouse fibroblasts for 3D bioprinting (3DBP), which is a reported bioink [184,185]. To reduce the probability of cell death during the encapsulation process, DeSimone et al. integrated the -RGD sequence onto the eADF4(C16) protein. The synthesized eADF4(C16)-RGD protein not only significantly enhances the proliferation and diffusion of BALB/3T3 cells but also exhibits higher long-term stability compared to most other bio-inks [186].

Inspired by the excellent mechanical properties of spider silk, Kuhbier et al. also prepared natural spider silk into microsurgical sutures, which have a maximum load and tensile strength that are significantly more than twice that of nylon sutures. These can be applied to

microsurgical nerve repair [187,188], flexor tendon repair [189], or the beauty industry [190].When orb-weaving spiders capture flying prey, they rotate their webs to counteract the mechanical energy produced by the flight. The realization of this function highly depends on the unique combination of tensile strength and elasticity of the spider silk, which can be utilized as structural materials for high-speed industries or for bulletproof vests, bulletproof vehicles, etc. [191,192]. Green optical waveguides prepared using recombinant spider silk protein eADF4(C16) as the cladding material can enhance the durable adhesion between the cellulose core and the spider silk cladding, possessing excellent mechanical and optical propagation properties. Their future application prospects in biomedical optical imaging and therapy are broad [193,194].

6. Conclusion

This study delves into the structure-activity relationship between spider silk protein motifs and mechanical properties, based on a systematic perspective of the structure-production-application. It innovatively integrates a multi-host expression system, including CRISPR technology-optimized transgenic plants and mammalian cells, with biomimetic spinning technology involving liquid crystal theory and liquid-liquid phase separation principles; it also demonstrates the cutting-edge application potential of spider silk materials in the fields of tissue engineering, targeted therapy, and emerging technologies. Furthermore, this study presents unique insights into the regulation of degradation rates, clinical translation pathways, and the expansion of environmental and military applications. It provides a systematic framework with scientific depth and engineering guidance value for the field of spider silk research.

Over the past decades, spider silk has garnered significant research attention owing to its exceptional mechanical properties, biodegradability, and biocompatibility. Spider silk, which participates in a range of physiological activities, serves as a crucial survival tool for spiders [19,20]. But spiders are aggressive, and if kept in limited space, they often kill each other. Therefore, researchers used various heterologous expression systems such as bacteria, plants, yeast, mammalian cell lines, and insects to produce recombinant spider silk proteins [1,70,195–197]. Although heterologous expression systems have made some progress in producing recombinant spider silk proteins, the cost is still relatively high compared to traditional synthetic polymers. Researchers are working hard to reduce production costs and improve expression efficiency by improving culture media, optimizing viral vectors, or using gene editing techniques such as CRISPR/Cas9 [72,75,76].

Currently, recombinant spider silk protein has emerged as a versatile material for developing novel biomaterials, encompassing high-strength fibers [198], bioadhesives [199], bioinks [200–202], and tissue engineering scaffolds [113]. These biomaterials exhibit promising potential across diverse applications, including advanced wound dressings, orthopedic implants for bone regeneration, and innovative strategies in cancer treatment [118–120,132–134,140–147]. Nonetheless, despite their remarkable strength and resilience, the artificial fibers face challenges in fully mimicking the intricate hierarchical architecture inherent in natural spider silk. Hence, in comparison to natural spider silk, artificial fibers exhibit a notable disparity in mechanical properties [161,198].

The application of spider silk biomaterials has been widely studied. When scaffold materials comprised of either natural or recombinant spider silk fibers are implanted into animals, the subsequent release of inflammatory cytokines has been observed, which not only promotes angiogenesis, nerve repair, and wound healing [130], but also renders them the material of choice for the construction of artificial skin, ligaments, tendons, bones, and various other tissues. In addition, the by-products produced during the degradation process of spider silk are harmless and can be recognized and cleared by the immune system and its related cells [9]. Sutures fabricated utilizing this property undergo gradual degradation post-surgery, thereby minimizing the necessity for secondary procedures and mitigating the risk of infection. Moreover, spider silk fibers boast a slow degradation rate, enabling them to maintain mechanical stability for extended periods under physiological conditions. This characteristic is particularly advantageous in drug delivery systems [150,164], as it ensures sustained release of therapeutic agents.

7. Future prospects of spider silk applications

Spider silk biomaterials still face several challenges in their widespread application in practical fields, despite exhibiting many outstanding characteristics. Firstly, the relatively high production cost of spider silk protein somewhat restricts its extensive application in the medical field. Scientists have achieved recombinant expression of spider silk protein through genetic engineering techniques, but production efficiency still requires further improvement [77–88]. Secondly, the fibrillation process of spider silk protein is relatively complex, necessitating stringent control of conditions to achieve the desired fiber structure and performance [24,63–65]. Although spider silk biomaterials demonstrate excellent performance in vitro experiments, in vivo studies on spider silk materials are currently limited, and how to transform them into clinically available products still requires extensive research by researchers. Researchers need to strive for sustainable production of spider silk fibers through genetic engineering and biosynthetic methods. Simultaneously, initiating further clinical trials is crucial to better utilize these materials in the biomedical field, ensuring their future success. Moreover, ensuring the in vivo safety and efficacy of spider silk materials remains a vital direction for future research.

The continuous development of genetic engineering, materials science, and biomedical technology, will further broaden the application prospects of spider silk biomaterials. Scientists will continue to develop more efficient and cost-effective production techniques for spider silk protein and optimize fibrillation processes. To make a greater contribution to human health, conducting thorough research on the biocompatibility and immunogenicity of spider silk protein will facilitate its extensive application in the medical field. Furthermore, the potential applications of spider silk biomaterials in environmental protection, textiles, and military fields warrant further investigation and development.

CRediT authorship contribution statement

Shangrong Hu: Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Sijing Wan:** Writing – review & editing, Project administration. **Xinyu Zhang:** Writing – original draft, Investigation. **Xianzhong Wang:** Visualization, Formal analysis. **Liwen Guan:** Investigation. **Yuxin Ge:** Investigation. **Yan Li:** Investigation. **Jianlin Luo:** Writing – review & editing, Project administration, Funding acquisition. **Bin Tang:** Visualization, Validation, Supervision, Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

References

[1] A. Rising, M. Widhe, J. Johansson, M. Hedhammar, Spider silk proteins: recent advances in recombinant production, structure-function relationships and biomedical applications, Cell. Mol. Life Sci. 68 (2) (2011) 169–184.

[2] H. Chung, T.Y. Kim, S.Y. Lee, Recent advances in production of recombinant spider silk proteins, Curr. Opin. Biotechnol. 23 (6) (2012) 957–964.

[3] E.S. Lintz, T.R. Scheibel, Dragline, egg stalk and byssus: a comparison of outstanding protein fibers and their potential for developing new materials, Adv. Funct. Mater. 23 (2013) 4467–4482.

[4] C.H. Bowen, B. Dai, C.J. Sargent, W. Bai, P. Ladiwala, H. Feng, W. Huang, D. L. Kaplan, J. MGalazka, F. Zhang, Recombinant spidroins fully replicate primary mechanical properties of natural spider silk, Biomacromolecules 19 (9) (2018) 3853–3860.

[5] J. Ruan, M. Wu, D. Zhao, X. Tang, B.Z. Hong, Improvement of mechanical properties of *Bombyx mori* silk based on Type1 sequence of MaSp1 from *Latrodectus hesperus*, Sci. Sericulture 47 (5) (2021) 0423–0429.

[6] A. Nova, S. Keten, N.M. Pugno, A. Redaelli, M.J. Buehler, Molecular and nanostructural mechanisms of deformation, strength and toughness of spider silk fibrils, Nano Lett. 10 (7) (2010) 2626–2634.

[7] M. Dong, Y. Guo, M. Wang, X. Hao, Research progress of bulletproof and stab-resistant high-performance fibers and composite materials, J. Cloth. Res. 9 (2) (2024).

[8] G.H. Altman, F. Diaz, T.C. Jakuba, T. Calabro, R.L. Horan, J. Chen, H. Lu, J. Richmond, D.L. Kaplan, Silk-based biomaterials, Biomaterials 24 (2003) 401–416.

[9] S. Salehi, K. Koeck, T. Scheibel, Spider silk for tissue engineering applications, Molecules 25 (3) (2020) 737.

[10] D. Stengel, J.B. Addison, D. Onofrei, G.N.U. Huynh, G. Youssef, G.P. Holland, Hydration-induced β-sheet crosslinking of α-helical-rich spider prey wrapping silk, Adv. Funct. Mater. 31 (2021) 1–10.

[11] S.J. Blamires, A. Rawal, A.D. Edwards, J.L. Yarger, S. Oberst, B.J. Allardyce, R. Rajkhowa, Methods for silk property analyses across structural hierarchies and scales, Molecules 28 (5) (2023) 2120.

[12] M. Ramezaniaghdam, N.D. Nahdi, R. Reski, Recombinant spider silk: promises and bottlenecks, Front. Bioeng. Biotechnol. 8 (10) (2022) 835637.

[13] J. Hou, G. Yang, D. Liu, J. Yang, Recombinant spider silk proteins:from structure, design to application, Chem. Life 40 (11) (2020) 2006–2013.

[14] Q. Jia, Q. Meng, *Araneus ventricosus* recombinant chimeric spidroin:a biomaterial with high molecular weight, J. Biol. 39 (1) (2022).

[15] L. Eisoldt, A. Smith, T. Scheibel, Decoding the secrets of spider silk, Mater. Today 14 (3) (2011) 80–86.

[16] A.P. Kiseleva, G.O. Kiselev, V.O. Nikolaeva, G. Seisenbaeva, V. Kessler, P. V. Krivoshapkin, E.F. Krivoshapkina, Hybrid spider silk with inorganic nanomaterials, Nanomaterials (Basel) 10 (9) (2020) 1853.

[17] P.L. Babb, M. Gregorič, N.F. Lahens, D.N. Nicholson, C.Y. Hayashi, L. Higgins, M. Kuntner, I. Agnarsson, B.F. Voight, Characterization of the genome and silk-gland transcriptomes of Darwin's bark spider (*Caerostris darwini*), PLoS One 17 (6) (2022) e0268660.

[18] M. Wang, Z. Yang, M. Si, Analyses of the Raman spectra and mechanical properties of major ampullate silks from two spiders, J. Light Scattering 35 (1) (2023).

[19] K.W. Sanggaard, J.S. Bechsgaard, X. Fang, J. Duan, T.F. Dyrlund, V. Gupta, X. Jiang, L. Cheng, D. Fan, Y. Feng, L. Han, Z. Huang, Z. Wu, L. Liao, V. Settepani, I.B. Thøgersen, B. Vanthournout, T. Wang, Y. Zhu, P. Funch, J.J. Enghild, L. Schauser, S.U. Andersen, P. Villesen, M.H. Schierup, T. Bilde, J. Wang, Spider genomes provide insight into composition and evolution of venom and silk, Nat. Commun. 5 (2014) 3765.

[20] W. Huang, Expression, Purification and Drug Loading of Recombinant Spider Silk Protein MaSp2(8R)+CT [D], Sichuan University of Science & Engineering, 2022.

[21] L. Xue, G. Chen, X. Zhang, Q. Meng, Cloning and expression and CD spectra of the N-terminal domain of flagelliform spidroin from *Araneus ventricosus*, Life Sci. Res. 21 (5) (2017).

[22] D.H. De Oliveira, V. Gowda, T. Sparrman, L. Gustafsson, R. Sanches Pires, C. Riekel, A. Barth, C. Lendel, M. Hedhammar, Structural conversion of the spidroin C-terminal domain during assembly of spider silk fibers, Nat. Commun. 15 (1) (2024) 4670.

[23] D. Bittencourt, P.F. Oliveira, F. Prosdocimi, E.L. Rech, Protein families, natural history and biotechnological aspects of spider silk, Genet. Mol. Res. 11 (3) (2012) 2360–2380.

[24] A.D. Malay, H.C. Craig, J. Chen, N.A. Oktaviani, K. Numata, Complexity of spider dragline silk, Biomacromolecules 23 (5) (2022) 1827–1840.

[25] T. Lefevre, M. Pézolet, Unexpected β-sheets and molecular orientation in flagelliformspider silk as revealed by Raman spectromicroscopy, Soft Matter 8 (2012) 6350–6635.

[26] O. Tokareva, M. Jacobsen, M. Buehler, J. Wong, D.L. Kaplan, Structure-function-property-design interplay in biopolymers: spider silk, Acta Biomater. 10 (4) (2014) 1612–1626.

[27] J.L. Yarger, B.R. Cherry, A. van der Vaart, Uncovering the structure-function relationship in spider silk, Nat. Rev. Mater. 3 (2018).

[28] E. Doblhofer, A. Heidebrecht, T. Scheibel, To spin or not to spin: spider silk fibers and more, Appl. Microbiol. Biotechnol. 99 (22) (2015) 9361–9380.

[29] H. Zhang, J. Liu, Characteristics and molecular engineering of spider dragline silk protein, Prog. Nat. Sci. 15 (6) (2005) 641–647.

[30] J.A. Kluge, O. Rabotyagova, G.G. Leiskx, D.L. Kaplan, Spider silks and their applications, Trends Biotechnol. 26 (5) (2008) 244–251.

[31] A. Heidebrecht, T. Scheibel, Recombinant production of spider silk proteins, Adv. Appl. Microbiol. 82 (2013) 115–153.

[32] A. Rising, J. Johansson, Toward spinning artificial spider silk, Nat. Chem. Biol. 11 (5) (2015) 309–315.

[33] N. Kong, F. Wan, W. Dai, P. Wu, C. Su, C. Peng, K. Zheng, X. Chen, S. Ling, J. Gong, Y. Yao, A cuboid spider silk: structure-function relationship and polypeptide signature, Macromol. Rapid Commun. 41 (6) (2020) e1900583.

[34] B. Lin, Y. Xiong, H. Chen, S. Wei, P. Ren, C. Cheng, B. He, Primary structure characterization and biosynthesis of spider silk proteins for multifunctional biomaterials, Chin. J. Biotechnol. 40 (3) (2024) 687–704.

[35] M.A. Collin, T.H. Clarke 3rd, N.A. Ayoub, C.Y. Hayashi, Genomic perspectives of spider silk genes through target capture sequencing: conservation of stabilization mechanisms and homology-based structural models of spidroin terminal regions, Int. J. Biol. Macromol. 113 (2018) 829–840.

[36] J. Gatesy, C. Hayashi, D. Motriuk, J. Woods, R. Lewis, Extreme diversity, conservation, and convergence of spider silk fibroin sequences, Science 291 (5513) (2001) 2603–2605.

[37] Y. Watanabe, K. Arakawa, Molecular mechanisms of the high performance of spider silks revealed through multi-omics analysis, Biophys. Physicobiol. 20 (1) (2023) e200014.

[38] J.E. Garb, R.A. Haney, R.A. Schwagerx, M. Gregorič, M. Kuntner, I. Agnarsson, T. A. Blackledge, The transcriptome of Darwin's bark spider silk glands predicts proteins contributing to dragline silk toughness, Commun. Biol. 2 (2019) 275.

[39] N. Kono, R. Ohtoshi, A.D. Malay, M. Mori, H. Masunaga, Y. Yoshida, H. Nakamura, K. Numata, K. Arakawa, Darwin's bark spider shares a spidroin repertoire with *Caerostris extrusa* but achieves extraordinary silk toughness through gene expression, Open Biol. 11 (12) (2021) 210242.

[40] I. Iachina, J. Fiutowski, H.G. Rubahn, F. Vollrath, J.R. Brewer, Nanoscale imaging of major and minor ampullate silk from the orb-web spider Nephila Madagascariensis, Sci. Rep. 13 (1) (Apr 24, 2023) 6695, https://doi.org/10.1038/s41598-023-33839-z.

[41] Z. Yang, G. Chen, Q. Meng, Expression of *Araneus ventricosus* minor ampullate spidroin, Chin. J. Biotechnol. 29 (9) (2013) 1323–1331.

[42] X. Peng, Z. Liu, J. Gao, Y. Zhang, H. Wang, C. Li, X. Lv, Y. Gao, H. Deng, B. Zhao, T. Gao, H. Li, Influence of spider silk protein structure on mechanical and biological properties for energetic material detection, Molecules 29 (5) (2024) 1025.

[43] J.M. Vienneau-Hathaway, E.R. Brassfield, A.K. Lane, M.A. Collin, S.M. Correa-Garhwal, T.H. Clarke, E.E. Schwager, J.E. Garb, C.Y. Hayashi, N.A. Ayoub, Duplication and concerted evolution of MiSp-encoding genes underlie the material properties of minor ampullate silks of cobweb weaving spiders, BMC Evol. Biol. 17 (1) (2017) 78.

[44] H. Yu, G. Chen, L. Li, G. Wei, Y. Li, S. Xiong, X. Qi, Spider minor ampullate silk protein nanoparticles: an effective protein delivery system capable of enhancing systemic immune responses, MedComm (2020) 5 (7) (Jun 15, 2024) e573, https://doi.org/10.1002/mco2.573.

[45] H. Nakamura, N. Kono, M. Mori, H. Masunaga, K. Numata, K. Arakawa, Composition of minor ampullate silk makes its properties different from those of major ampullate silk, Biomacromolecules 24 (5) (May 8, 2023) 2042–2051, https://doi.org/10.1021/acs.biomac.2c01474.

[46] L. Römer, T. Scheibel, The elaborate structure of spider silk: structure and function of a natural high performance fiber, Prion 2 (4) (2008) 154–161.

[47] S. Xu, X. Li, Y. Zhou, Y. Lin, Q. Meng, Structural characterization and mechanical properties of chimeric Masp1/Flag minispidroins, Biochimie 168 (2020) 251–258.

[48] D.M. de C. Bittencourt, P.F. Oliveira, B.M. Souto, S.M. de Freitas, L.P. Silva, A. M. Murad, V.A. Michalczechen-Lacerda, R.V. Lewis, E.L. Rech, Molecular dynamics of synthetic flagelliform silk fiber assembly, Macromol. Mater. Eng. 306 (1) (2021) 2000530.

[49] C.Y. Hayashi, R.V. Lewis, Spider flagelliform silk: lessons in protein design, gene structure, and molecular evolution, Bioessays 23 (8) (2001) 750–756.

[50] D. Yang, S. Wang, K. Wang, S. Zheng, X. Zan, R. Wen, Physical properties of the second type of aciniform spidroin (AcSp2) from *Neoscona theisi* reveal a pH-dependent self-assembly repetitive domain, ACS Biomater. Sci. Eng. 9 (12) (2023) 6670–6682.

[51] C.Y. Hayashi, T.A. Blackledge, R.V. Lewis, 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 (2004) 1950–1959.

[52] N.A. Ayoub, N.A. Garbx, A. Kuelbs, C.Y. Hayashi, Ancient properties of spider silks revealed by the complete gene sequence of the prey-wrapping silk protein (AcSp1), Mol. Biol. Evol. 30 (3) (2013) 589–601.

[53] R. Wen, K. Wang, D. Yang, T. Yu, X. Zan, Q. Meng, The novel aciniform silk protein (AcSp2-v2) reveals the unique repetitive domain with high acid and thermal stability and self-assembly capability, Int. J. Biol. Macromol. 202 (2022) 91–101.

[54] P.L. Babb, N.F. Lahens, S.M. Correa-Garhwal, D.N. Nicholson, E.J. Kim, J. B. Hogenesch, M. Kuntner, L. Higgins, C.Y. Hayashi, I. Agnarsson, B.F. Voight, The *Nephila clavipes* genome highlights the diversity of spider silk genes and their complex expression, Nat. Genet. 49 (6) (2017) 895–903.

[55] N. Yu, Z. Yang, Z. Fan, Z. Liu, Classification and functional characterization of spidroin genes in a wandering spider, *Pardosa pseudoannulata*, Insect Biochem. Mol. Biol. 151 (2022) 103862.

[56] R. Wen, K. Wang, Q. Meng, Two novel tubuliform silk gene sequences from *Araneus ventricosus* provide evidence for multiple loci in genome, Int. J. Biol.

Macromol. 160 (Oct 1, 2020) 806–813, https://doi.org/10.1016/j.ijbiomac.2020.05.141.
[57] R. Wen, X. Liu, Q. Meng, Characterization of full-length tubuliform spidroin gene from *Araneus ventricosus*, Int. J. Biol. Macromol. 105 (Pt 1) (2017) 702–710.
[58] R.C. Chaw, M. Collin, M. Wimmer, K.L. Helmrick, C.Y. Hayashi, Egg case silk gene sequences from *Argiope* spiders: evidence for multiple loci and a loss of function between paralogs, G3 (Bethesda) 8 (1) (2018) 231–238.
[59] J.O. Wolff, I. Grawe, M. Wirth, A. Karstedt, S.N. Gorb, Spider's super-glue: thread anchors are composite adhesives with synergistic hierarchical organization, Soft Matter 11 (12) (2015) 2394–2403.
[60] K. Wang, R. Wen, Q. Jia, X. Liu, J. Xiao, Q. Meng, Analysis of the full-length pyriform spidroin gene sequence, Genes (Basel) 10 (6) (2019) 425.
[61] H. Zhu, A. Rising, J. Johansson, X. Zhang, Y. Lin, L. Zhang, T. Yi, J. Mi, Q. Meng, Tensile properties of synthetic pyriform spider silk fibers depend on the number of repetitive units as well as the presence of N- and C-terminal domains, Int. J. Biol. Macromol. 154 (2020) 765–772.
[62] K. Wang, R. Wen, S. Wang, L. Tian, J. Xiao, Q. Meng, The molecular structure of novel pyriform spidroin (PySp2) reveals extremely complex central repetitive region, Int. J. Biol. Macromol. 145 (2020) 437–444.
[63] T.Y. Lin, H. Masunaga, R. Sato, A.D. Malay, K. Toyooka, T. Hikima, K. Numata, Liquid crystalline granules align in a hierarchical structure to produce spider dragline microfibrils, Biomacromolecules 18 (4) (2017) 1350–1355.
[64] M. Andersson, G. Chen, M. Otikovs, M. Landrehx, K. Nordling, N. Kronqvist, P. Westermark, H. Jörnvall, S. Knight, Y. Ridderstråle, L. Holm, Q. Meng, K. Jaudzems, M. Chesler, J. Johansson, A. Rising, Carbonic anhydrase generates CO_2 and H+ that drive spider silk formation via opposite effects on the terminal domains, PLoS Biol. 12 (8) (2014) e1001921.
[65] J. Sparkes, C. Holland, Analysis of the pressure requirements for silk spinning reveals a pultrusion dominated process, Nat. Commun. 8 (1) (2017) 594.
[66] V.T. Trossmann, S. Lentz, T. Scheibel, Factors influencing properties of spider silk coatings and their interactions within a biological environment, J. Funct. Biomater. 14 (8) (2023) 434.
[67] S.R. Fahnestock, S.L. Irwin, Synthetic spider dragline silk proteins and their production in *Escherichia* coli, Appl. Microbiol. Biotechnol. 47 (1) (1997) 23–32.
[68] Q. Jin, F. Pan, C.F. Hu, S.Y. Lee, X.X. Xia, Z.G. Qian, Secretory production of spider silk proteins in metabolically engineered *Corynebacterium glutamicum* for spinning into tough fibers, Metab. Eng. 70 (2022) 102–114.
[69] A. Connor, R.H. Zha, M. Kofas, Production and secretion of recombinant spider silk in *Bacillus megaterium*, Microb. Cell Factories 23 (1) (2024) 35.
[70] S.R. Fahnestock, L.A. Bedzyk, Production of synthetic spider dragline silk protein in *Pichia pastoris*, Appl. Microbiol. Biotechnol. 47 (1997) 33–39.
[71] D. Huemmerich, T. Scheibel, V. Follrath, S. Cohen, U. Gat, S. Ittah, Novel assembly properties of recombinant spider dragline silk proteins, Curr. Biol. 14 (22) (2004) 2070–2074.
[72] Y. Zhang, J. Hu, Y. Miao, A. Zhao, T. Zhao, D. Wu, L. Liang, A. Miikura, K. Shiomi, Z. Kajiura, M. Nakagaki, Expression of EGFP-spider dragline silk fusion protein in BmN cells and larvae of silkworm showed the solubility is primary limit for dragline proteins yield, Mol. Biol. Rep. 35 (2008) 329–335.
[73] H. Wen, X. Lan, Y. Zhang, T. Zhao, Y. Wang, Z. Kajiura, M. Nakagakiv, Transgenic silkworms (*Bombyx mori*) produce recombinant spider dragline silk in cocoons, Mol. Biol. Rep. 37 (2010) 1815–1821.
[74] Y. Wang, Z. Li, J. Xu, B. Zeng, L. Ling, L. You, Y. Chen, Y. Huang, A. Tan, The CRISPR/Cas system mediates efficient genome engineering in *Bombyx mori*, Cell Res. 23 (12) (2013) 1414–1416.
[75] J. Xu, Q. Dong, Y. Yu, B. Niu, D. Ji, M. Li, Y. Huang, X. Chen, A. Tan, Mass spider silk production through targeted gene replacement in *Bombyx mori*, Proc. Natl. Acad. Sci. USA 115 (35) (2018) 8757–8762.
[76] X. Zhang, L. Xia, B.A. Day, H.i. Harris, P. Oliveira, C. Knittel, A.L. Licon, C. Gong, G. Dion, R.V. Lewis, J.A. Jones, CRISPR/Cas9 initiated transgenic silkworms as a natural spinner of spider silk, Biomacromolecules 20 (6) (2019) 2252–2264.
[77] D.R. Whittall, K.V. Baker, R. Breitling, E. Takano, Host systems for the production of recombinant spider silk, Trends Biotechnol. 39 (6) (2021) 560–573.
[78] P. Aramwit, Q. Jiang, S. Muppuri, N. Reddy, Transgenic modifications of silkworms as a means to obtain therapeutic biomolecules and protein fibers with exceptional properties, Biotechnol. Bioeng. 120 (10) (2023) 2827–2839.
[79] A. Lazaris, A. Arcidiaconox, Y. Huang, J.F. Zhou, F. Duguay, N. Chretien, E. A. Welsh, J.W. Soares, C.N. Karatzas, Spider silk fibers spun from soluble recombinant silk produced in mammalian cells, Science 295 (5554) (2002) 472–476.
[80] R.F. Service, Materials science. Mammalian cells spin a spidery new yarn, Science 295 (5554) (2002) 419–421.
[81] D. Williams, Sows' ears, silk purses and goats' milk: new production methods and medical applications for silk, Med. Device Technol. 14 (5) (2003) 9–11.
[82] H.T. Xu, B.L. Fan, S.Y. Yu, Y.H. Huang, Z.H. Zhao, Z.X. Lian, Y.P. Dai, L.L. Wang, Z.L. Liu, J. Fei, N. Li, Construct synthetic gene encoding artificial spider dragline silk protein and its expression in milk of transgenic mice, Anim. Biotechnol. 18 (1) (2007) 1–12.
[83] H. Poddar, R. Breitling, E. Takano, Towards engineering and production of artificial spider silk using tools of synthetic biology, Eng. Biol. 4 (1) (2020) 1–6.
[84] J.V.D. Molino, T.L. Alves, L.S. Ferreira-Camargo, M.A. Croce, A. Tanaka, F.X. Buson, P. dFRibeiro, A.B. Campos-Salazar, E. Antonio, A.S. Maizel, V.M. Siratuti, C. Costa, S.R. Wlodarczyk, et al., Chimeric spider silk production in microalgae: a modular bionanomaterial, Res. Ideas Outcomes. 2, e9342.
[85] J.E. Park, Y.J. Jeong, J.B. Park, H.Y. Kim, Y.H. Yoo, K.S. Lee, W.T. Yang, D. H. Kim, J.M. Kim, Dietary exposure to transgenic rice expressing the spider silk protein fibroin reduces blood glucose levels in diabetic mice: the potential role of insulin receptor substrate-1 phosphorylation in adipocytes, Dev. Reprod. 23 (3) (2019) 223–229.
[86] J.T. Prince, K.P. McGrath, C.M. DiGirolamo, D.L. Kaplan, Construction, cloning, and expression of synthetic genes encoding spider dragline silk, Biochemistry 34 (34) (1995) 10879–10885.
[87] F. Teulé, A.R. Cooper, W.A. Furin, D. Bittencourt, E.L. Rech, A. Brooks, R. V. Lewis, A protocol for the production of recombinant spider silk-like proteins for artificial fiber spinning, Nat. Protoc. 4 (3) (2009) 341–355.
[88] J. Scheller, K.H. Gührs, F. Grosse, U. Conrad, Production of spider silk proteins in tobacco and potato, Nat. Biotechnol. 19 (6) (2001) 573–577.
[89] C.A. Peng, J. Russo, C. Gravgaard, H. McCartney, W. Gaines, W.R. Marcotte Jr., Spider silk-like proteins derived from transgenic *Nicotiana tabacum*, Transgenic Res. 25 (4) (2016) 517–526.
[90] C.A. Peng, L. Kozubowski, W.R. Marcotte Jr., Advances in plant-derived scaffold proteins, Front. Plant Sci. 11 (2020) 122.
[91] J. Yang, L.A. Barr, S.R. Fahnestock, Z.B. Liu, High yield recombinant silk-like protein production in transgenic plants through protein targeting, Transgenic Res. 14 (3) (2005) 313–324.
[92] V. Hauptmann, N. Weichert, M. Menzel, D. Knoch, N. Paege, J. Scheller, U. Spohn, U. Conrad, M. Gils, Native-sized spider silk proteins synthesized *in planta* via intein-based multimerization, Transgenic Res. (2013) 369–377.
[93] N. Weichert, V. Hauptmann, M. Menzel, K. Schallau, P. Gunkel, T.C. Hertel, M. Pietzsch, U. Spohn, U. Conrad, Transglutamination allows production and characterization of native-sized ELPylated spider silk proteins from transgenic plants, Plant Biotechnol. J. 12 (2) (2014) 265–275.
[94] C. Thagun, T. Suzuki, Y. Kodama, K. Numata, C-Terminal domain controls protein quality and secretion of spider silk in tobacco cells, Adv. Biol (Weinh.) (12) (Dec 7, 2023) e2300011.
[95] T.B. Aigner, E. DeSimone, T. Scheibel, Biomedical applications of recombinant silk-based materials, Adv. Mater. Weinheim 30 (2018) e1704636.
[96] A. Saez, M. Ghibaudo, A. Buguin, P. Silberzan, B. Ladoux, Rigidity-driven growth and migration of epithelial cells on microstructured anisotropic substrates, Proc. Natl. Acad. Sci. USA 104 (20) (2007) 8281–8286.
[97] R.G. Wells, The role of matrix stiffness in regulating cell behavior, Hepatology 47 (4) (2008) 1394–1400.
[98] E. Doblhofer, T. Scheibel, Engineering of recombinant spider silk proteins allows defined uptake and release of substances, J. Pharm. Sci. 104 (3) (2015) 988–994.
[99] F. Berthiaume, T.J. Maguire, M.L. Yarmush, Tissue engineering and regenerative medicine: history, progress, and challenges, Annu. Rev. Chem. Biomol. Eng. 2 (2011) 403–430.
[100] E. Cosgriff-Hernandez, E. Timminsx, Model-directed design of tissue engineering scaffolds, ACS Biomater. Sci. Eng. 8 (11) (2022) 4622–4624.
[101] M.P. Nikolova, M.S. Chavali, Recent advances in biomaterials for 3D scaffolds: a review, Bioact. Mater. 4 (2019) 271–292.
[102] Q. Zhang, M. Li, W. Hu, X. Wang, J. Hu, Spidroin-based biomaterials in tissue engineering: general approaches and potential stem cell therapies, Stem Cells Int. 2021 (2021) 7141550.
[103] B.N. Brown, S.F. Badylak, Extracellular matrix as an inductive scaffold for functional tissue reconstruction, Transl. Res. 163 (4) (2014) 268–285.
[104] R.J. Hickey, A.E. Pelling, Cellulose biomaterials for tissue engineering, Front. Bioeng. Biotechnol. 7 (2019) 45.
[105] A.E. Kramerx, T.B. Aigner, J. Petzold, J. Roshanbinfarx, T. Scheibel, F.B. Engel, Recombinant spider silk protein eADF4(C16)-RGD coatings are suitable for cardiac tissue engineering, Sci. Rep. 10 (1) (2020) 8789.
[106] K.M. Moore, A.B. Murthy, E.G. Graham-Gurysh, S.D. Hingtgen, E.M. Bachelder, K. M. Ainslie, Polymeric biomaterial scaffolds for tumoricidal stem cell glioblastoma therapy, ACS Biomater. Sci. Eng. 6 (7) (2020) 3762–3777.
[107] K.Y. Lee, D.J. Mooney, Alginate: properties and biomedical applications, Prog. Polym. Sci. 37 (1) (2012) 106–126.
[108] P. Siqueira, É. Siqueira, A.E. de Lima, G. Siqueira, A.D. Pinzón-Garcia, A.P. Lopes, M.E.C. Segura, A. Isaac, F.V. Pereira, V.R. Botaro, Three-dimensional stable alginate-nanocellulose gels for biomedical applications: towards tunable mechanical properties and cell growing, Nanomaterials (Basel) 9 (1) (2019) 78.
[109] S. Fooladi, M.H. Nematollahi, N. Rabiee, S. Iravani, Bacterial cellulose-based materials: a perspective on cardiovascular tissue engineering applications, ACS Biomater. Sci. Eng. 9 (6) (2023) 2949–2969.
[110] A. Ressler, Chitosan-based biomaterials for bone tissue engineering applications: a short review, Polymers (Basel) 14 (16) (2022) 3430.
[111] W. Sun, D.A. Gregory, M.A. Tomeh, X. Zhao, Silk fibroin as a functional biomaterial for tissue engineering, Int. J. Mol. Sci. 22 (3) (2021) 1499.
[112] Z. Chen, Y. Fu, X. Zhang, Y. Xuan, Research progress in degradation behavior and applications of PLGA, China Plastics (2014) 1542–1557.
[113] D. Steiner, G. Lang, L. Fischer, S. Winkler, T. Fey, P. Greil, T. Scheibel, R.E. Horch, A. Arkudas, Intrinsic vascularization of recombinant eADF4(C16) spider silk matrices in the arteriovenous loop model, Tissue Eng. Part A 25 (21−22) (2019) 1504–1513.
[114] L. Sun, X. Li, T. Yang, P. Lu, P. Du, C. Jing, Z. Chen, F. Lin, G. Zhao, L. Zhao, Construction of spider silk protein small-caliber tissue engineering vascular grafts based on dynamic culture and its performance evaluation, J. Biomed. Mater. Res. A 111 (1) (2023) 71–87.
[115] W. Chen, K. Liu, X. Liao, J. Wu, L. Chen, Z. Yang, X. Wang, Y. Liao, G. Fu, X. Yang, Z. Wang, G. Qu, L. Wang, Y. Zhou, Z. Zhang, C. Yang, S. Ni, J. Zheng, T.H. Tao, D. Zou, Harmonizing thickness and permeability in bone tissue engineering: a novel silk fibroin membrane inspired by spider silk dynamics, Adv. Mater. 36 (13) (2024) e2310697.

[116] N. Johari, A. Khodaei, A. Samadikuchaksaraei, R.L. Reis, S.C. Kundu, L. Moroni, Ancient fibrous biomaterials from silkworm protein fibroin and spider silk blends: biomechanical patterns, Acta Biomater. 153 (2022) 38–67.
[117] J. Petzold, T.B. Aigner, F. Touska, K. Zimmermann, T. Scheibel, F.B. Engel, Surface features of recombinant spider silk protein eADF4(κ16)-made materials are well-suited for cardiac tissue engineering, Adv. Funct. Mater. 27 (36) (2017).
[118] P.H. Zeplin, N.C. Maksimovikj, M.C. Jordan, J. Nickel, G. Lang, A.H. Leimer, L. Römer, T. Scheibel, Spider silk coatings as a bioshield to reduce periprosthetic fibrous capsule formation, Adv. Funct. Mater. 24 (18) (2014) 2658–2666.
[119] T.U. Esser, V.T. Trossmann, S. Lentz, F.B. Engel, T. Scheibel, Designing of spider silk proteins for human induced pluripotent stem cell-based cardiac tissue engineering, Mater. Today Bio 11 (2021) 100114.
[120] J.G. Hardy, J.G. Torres-Rendon, A. Leal-Egaña, A. Walther, H. Schlaad, H. Cölfenx, T.R. Scheibel, Biomineralization of engineered spider silk protein-based composite materials for bone tissue engineering, Materials (Basel) 9 (7) (2016) 560.
[121] T. Kokubo, S. Yamaguchi, Growth of novel ceramic layers on metals via chemical and heat treatments for inducing various biological functions, Front. Bioeng. Biotechnol. 3 (2015) 176.
[122] N. Imagawa, K. Inoue, K. Matsumoto, M. Omori, K. Yamamoto, Y. Nakajima, N. Kato-Kogoe, H. Nakano, P. Thi Minh Le, S. Yamaguchi, T. Ueno, Histological evaluation of porous additive-manufacturing titanium artificial bone in rat calvarial bone defects, Materials (Basel) 14 (18) (2021) 5360.
[123] K. Bavya Devi, V. Lalzawmliana, M. Saidivya, V. Kumar, M. Roy, S. Kumar Nandi, Magnesium phosphate bioceramics for bone tissue engineering, Chem. Rec. 22 (11) (2022) e202200136.
[124] R. Ma, T. Tang, Current strategies to improve the bioactivity of PEEK, Int. J. Mol. Sci. 15 (4) (2014) 5426–5445.
[125] M. Papadaki, T. Mahmood, P. Gupta, M.B. Claase, D.W. Grijpma, J. Riesle, C. Avan Blitterswijk, R. Langer, The different behaviors of skeletal muscle cells and chondrocytes on PEGT/PBT block copolymers are related to the surface properties of the substrate, J. Biomed. Mater. Res. 54 (1) (2001) 47–58.
[126] M.B. Claase, M.B. Olde Riekerink, J.D. de Bruijn, D.W. Grijpma, G.H. Engbers, J. Feijen, Enhanced bone marrow stromal cell adhesion and growth on segmented poly(ether ester)s based on poly(ethylene oxide) and poly(butylene terephthalate), Biomacromolecules 4 (1) (2003) 57–63, https://doi.org/10.1021/bm0256045.
[127] N. Dinjaski, R. Plowright, S. Zhou, D.J. Belton, C.C. Perry, D.L. Kaplan, Osteoinductive recombinant silk fusion proteins for bone regeneration, Acta Biomater. 49 (2017) 127–139.
[128] A.J. Mieszawska, L.D. Nadkarni, C.C. Perry, D.L. Kaplan, Nanoscale control of silica particle formation via silk-silica fusion proteins for bone regeneration, Chem. Mater. 22 (20) (2010) 5780–5785.
[129] D. Chouhan, B.B. Mandal, Silk biomaterials in wound healing and skin regeneration therapeutics: from bench to bedside, Acta Biomater. 103 (2020) 24–51.
[130] C. Liebsch, V. Bucan, B. Menger, F. Köhne, K.H. Waldmann, D. Vaslaitis, P. M. Vogt, S. Strauss, J.W. Kuhbier, Preliminary investigations of spider silk in wounds in vivo - implications for an innovative wound dressing, Burns 44 (7) (2018) 1829–1838.
[131] A. Steins, P. Dik, W.H. Müller, S.J. Vervoort, K. Reimers, J.W. Kuhbier, P.M. Vogt, A.A. van Apeldoorn, P.J. Coffer, K. Schepers, In vitro evaluation of spider silk meshes as a potential biomaterial for bladder reconstruction, PLoS One 10 (12) (2015) e0145240.
[132] D. Chouhan, N. Thatikonda, L. Nilebäck, M. Widhe, M. Hedhammar, B.B. Mandal, Recombinant spider silk functionalized silkworm silk matrices as potential bioactive wound dressings and skin grafts, ACS Appl. Mater. Interfaces 10 (28) (2018) 23560–23572.
[133] D. Chouhan, T.U. Lohe, N. Thatikonda, V. Naidu, M. Hedhammar, B.B. Mandal, Silkworm silk scaffolds functionalized with recombinant spider silk containing a fibronectin motif promotes healing of full-thickness burn wounds, ACS Biomater Sci. Eng. 5 (9) (2019) 4634–4645.
[134] D. Chouhan, P. Das, N. Thatikonda, S.K. Nandi, M. Hedhammar, M. Mandalx, Silkworm silk matrices coated with functionalized spider silk accelerate healing of diabetic wounds, ACS Biomater. Sci. Eng. 5 (7) (2019) 3537–3548.
[135] N. Kamble, D. Shukla, D. Bhat, Peripheral nerve injuries: electrophysiology for the neurosurgeon, Neurol. India 67 (6) (2019) 1419–1422.
[136] T. Gordon, Peripheral nerve regeneration and muscle reinnervation, Int. J. Mol. Sci. 21 (22) (2020) 8652.
[137] M. Modrak, M.A.H. Talukder, K. Gurgenashvili, M. Noble, J.C. Elfar, Peripheral nerve injury and myelination: potential therapeutic strategies, J. Neurosci. Res. 98 (5) (2020) 780–795.
[138] M.L. Hansson, U. Chatterjee, J. Francis, T. Arndt, C. Broman, J. Johansson, M. K. Sköld, A. Rising, Artificial spider silk supports and guides neurite extension in vitro, FASEB J. 35 (11) (2021) e21896.
[139] F. Millesi, T. Weiss, A. Mann, M. Haertinger, L. Semmler, P. Supper, D. Pils, A. Naghilou, C. Radtke, Defining the regenerative effects of native spider silk fibers on primary Schwann cells, sensory neurons, and nerve-associated fibroblasts, FASEB J. 35 (2) (2021) e21196.
[140] C. Allmeling, A. Jokuszies, K. Reimers, S. Kall, P.M. Vogt, Use of spider silk fibres as an innovative material in a biocompatible artificial nerve conduit, J. Cell. Mol. Med. 10 (3) (2006) 770–777.
[141] M.B. Chen, F. Zhang, W.C. Lineaweaver, Luminal fillers in nerve conduits for peripheral nerve repair, Ann. Plast. Surg. 57 (4) (2006) 462–471.
[142] A.I. Gravvanis, A.A. Lavdas, A. Papalois, D.A. Tsoutsos, R. Matsas, The beneficial effect of genetically engineered Schwann cells with enhanced motility in peripheral nerve regeneration: review, Acta Neurochir. Suppl. 100 (2007) 51–56.
[143] C. Allmeling, A. Jokuszies, K. Reimers, S. Kall, C.Y. Choi, G. Brandes, C. Kasper, T. Scheper, G. Muggenheim, P.M. Vogt, Spider silk fibres in artificial nerve constructs promote peripheral nerve regeneration, Cell Prolif. 41 (3) (2008) 408–420.
[144] F. Koop, S. Strauß, S. Peckx, T. Aper, M. Wilhelmi, C. Hartmann, J. Hegermann, J. Schipke, P.M. Vogt, V. Bucan, Preliminary application of native *Nephila edulis* spider silk and fibrin implant causes granulomatous foreign body reaction in vivo in rat's spinal cord, PLoS One 17 (3) (2022) e0264486.
[145] A. Naghilou, K. Peter, F. Millesi, S. Stadlmayr, S. Wolf, A. Rad, A. Semmlerx, A. Supperx, L. Ploszczanski, J. Liu, M. Burghammer, M. Riekel, A. Bismarck, E.H. G. Backus, H. Lichtenegger, C. Radtke, Insights into the material properties of dragline spider silk affecting Schwann cell migration, Int. J. Biol. Macromol. 244 (2023) 125398.
[146] C. Radtke, C. Allmeling, K.H. Waldmann, K.H. Reimersx, K. Thies, H.C. Schenk, A. Hillmer, M. Guggenheim, G. Brandes, G. Vogt, Spider silk constructs enhance axonal regeneration and remyelination in long nerve defects in sheep, PLoS One 6 (2) (2011) e16990.
[147] T. Kornfeld, T. Nesslerx, C. Helmer, C. Hannemannx, K.H. Waldmann, K.H. Peckx, P. Hoffmann, P. Brandesx, P.M. Vogt, C. Radtke, Spider silk nerve graft promotes axonal regeneration on long distance nerve defect in a sheep model, Biomaterials 271 (2021) 120692.
[148] T. Kornfeld, V.P. Mogt, V. Bucan, C.T. Peck, K. Reimers, C. Radtke, Characterization and Schwann cell seeding of up to 15.0 cm long spider silk nerve conduits for reconstruction of peripheral nerve defects, J. Funct. Biomater. 7 (4) (2016) 30.
[149] L. Semmler, A. Naghilou, F. Millesi, S. Wolf, A. Mann, S. Stadlmayr, S. Mero, L. Ploszczanski, L. Greutter, A. Woehrer, E. Placheta-Györi, V. Follrath, T. Weiss, C. Radtke, Silk-in-silk nerve guidance conduits enhance regeneration in a rat sciatic nerve injury model, Adv. Healthc. Mater. 12 (11) (2023) e2203237.
[150] A. Lammel, M. Schwab, M. Hofer, G. Winter, T. Scheibel, Recombinant spider silk particles as drug delivery vehicles, Biomaterials 32 (8) (2011) 2233–2240.
[151] Z. Chen, Z. Yex, H. Wang, Y. Yang, J. Cheng, H. Zhou, Y. Liu, Research progress of silk fibroin-based nanoparticulate drug delivery systems, Acta Pharm. Sin. 57 (6) (2022) 1792–1800.
[152] X. Wang, M. Li, J. Zhang, H. Gao, G. Yang, B. Ding, Progress on application of silk fibroin nanocarriers in oncology drug delivery system, Chin. Pharm. J. 59 (5) (2024).
[153] M. Branković, F. Zivic, N. Grujovic, I. Stojadinovic, S. Milenkovic, N. Kotorcevic, Review of spider silk applications in biomedical and tissue engineering, Biomimetics (Basel) 9 (3) (2024) 169.
[154] J.K. Vasir, K. Tambwekar, S. Garg, Bioadhesive microspheres as a controlled drug delivery system, Int. J. Pharm. 255 (1–2) (2003) 13–32.
[155] J.G. Hardy, A. Leal-Egaña, T.R. Scheibel, Engineered spider silk protein-based composites for drug delivery, Macromol. Biosci. 13 (10) (2013) 1431–1437.
[156] M.B. Schierling, E. Doblhofer, T. Scheibel, Cellular uptake of drug loaded spider silk particles, Biomater. Sci. 4 (10) (2016) 1515–1523.
[157] F. Mottaghitalab, M. Kiani, M. Farokhix, M. Kundux, M. Reisx, M. Gholami, H. Bardania, R. Dinarvand, P. Geramifar, D. Beiki, D. Atyabix, Targeted delivery system based on gemcitabine-loaded silk fibroin nanoparticles for lung cancer therapy, ACS Appl. Mater. Interfaces 9 (37) (2017) 31600–31611.
[158] K. Jastrzebska, E. Felcyn, M. Kozak, M. Szybowiczx, T. Buchwald, Z. Pietralik, T. Jesionowski, A. Mackiewicz, H. Dams-Kozlowska, The method of purifying bioengineered spider silk determines the silk sphere properties, Sci. Rep. 6 (2016) 28106.
[159] J. Pérez-Rigueiro, M. Elices, G.R. Plaza, G.V. Guinea, Basic principles in the design of spider silk fibers, Molecules 26 (6) (2021) 1794.
[160] D. Perera, L. Li, C. Walsh, J. Silliman, Y. Xiong, Q. Wang, Q. Schnieppx, Natural spider silk nanofibrils produced by assembling molecules or disassembling fibers, Acta Biomater. 168 (2023) 323–332.
[161] C. Belbéoch, J. Lejeune, P. Vromanc, P. Salaünx, Silkworm and spider silk electrospinning: a review, Environ. Chem. Lett. 19 (2) (2021) 1737–1763.
[162] S. Gao, Y. Xu, X. Li, Advances in silk fibroin-based novel drug delivery systems, Pharm. Clin. Res. 5 (2021).
[164] H.M. Herold, A. Döbl, S. Wohlrab, M. Humenik, T. Scheibel, Designed spider silk-based drug carrier for redox- or pH-triggered drug release, Biomacromolecules 21 (12) (2020) 4904–4912.
[165] V. Humenikx, V. Magdeburgx, T. Scheibel, Influence of repeat numbers on self-assembly rates of repetitive recombinant spider silk proteins, J. Struct. Biol. 186 (3) (2014) 431–437.
[166] V. Humenikx, M. Mohrandx, V. Scheibelx, Self-assembly of spider silk-fusion proteins comprising enzymatic and fluorescence activity, Bioconjug. Chem. 29 (4) (2018) 898–904.
[167] V. Hovanová, V. Hovanx, V. Humenikx, V. Sedlákx, Only kosmotrope anions trigger fibrillization of the recombinant core spidroin eADF4(C16) from *Araneus diadematus*, Protein Sci. 32 (12) (2023) e4832.
[168] A. Lammel, M. Schwab, U. Slotta, G. Winter, T. Scheibel, Processing conditions for the formation of spider silk microspheres, ChemSusChem 1 (5) (2008) 413–416.
[169] J. Chen, J. Hu, P. Zuo, X. Su, Z. Liu, M. Yang, Tailor-made spider-eggcase-silk spheres for efficient lysosomal drug delivery, RSC Adv. 8 (17) (2018) 9394–9401.
[171] K. Spiess, R. Enex, C.D. Keenan, J. Senker, F. Kremerb, T. Scheibel, Impact of initial solvent on thermal stability and mechanical properties of recombinant spider silk films, J. Mater. Chem. 21 (2011) 13594–13604.

[172] S. Wohlrab, K. Spieß, T. Scheibel, Varying surface hydrophobicities of coatings made of recombinant spider silk proteins, J. Mater. Chem. 22 (2012) 22050–22054.
[173] D. Huemmerich, U. Slotta, U. Scheibelx, Processing and modification of films made from recombinant spider silk proteins, Appl. Phys. A Mater. Sci. Process. 82 (2006) 219–222.
[174] E. Metwalli, U. Slotta, C. Darko, S.V. Roth, T. Scheibel, C.M. Papadakis, Structural changes of thin films from recombinant spider silk proteins upon post-treatment, Appl. Phys. A Mater. Sci. Process. 89 (2007) 655–661.
[175] E. Agostini, G. Winter, J. Engert, Water-based preparation of spider silk films as drug delivery matrices, J. Control. Release 213 (2015) 134–141.
[176] M. Lucke, I. Mottas, T. Herbst, C. Hotz, L. Römer, M. Schierling, H.M. Herold, U. Slotta, T. Spinetti, T. Scheibel, G. Winter, C. Bourquin, J. Engert, Engineered hybrid spider silk particles as delivery system for peptide vaccines, Biomaterials 172 (2018) 105–115.
[177] B. Bakhshandeh, S.S. Nateghi, M.M. Gazani, Z. Dehghani, F. Mohammadzadeh, A review on advances in the applications of spider silk in biomedical issues, Int. J. Biol. Macromol. 192 (2021) 258–271.
[178] P. Mulinti, J. Shreffler, R. Hasan, M. Dea, A.E. Brooks, Infection responsive smart delivery of antibiotics using recombinant spider silk nanospheres, Pharmaceutics 13 (9) (2021) 1358.
[179] K. Koh, J.K. Wang, J.X.Y. Chen, S.H. Hiew, H.S. Cheng, B. Gabryelczyk, M.I. G. Vos, Y.S. Yip, L. Chen, R.M. Sobota, D.K.K. Chua, N.S. Tan, C.Y. Tay, A. Miserez, Squid suckerin-spider silk fusion protein hydrogel for delivery of mesenchymal stem cell secretome to chronic wounds, Adv. Healthc. Mater. 12 (1) (2023) e2201900.
[180] W. Zhu, X. Ma, M. Gou, D. Mei, K. Zhang, S. Chen, 3D printing of functional biomaterials for tissue engineering, Curr. Opin. Biotechnol. 40 (Aug 2016) 103–112, https://doi.org/10.1016/j.copbio.2016.03.014.
[181] S. Chawla, S. Midha, A. Sharma, S. Ghosh, Silk-based bioinks for 3D bioprinting, Adv. Healthc. Mater. 7 (8) (Apr 2018) e1701204, https://doi.org/10.1002/adhm.201701204.
[182] M.K. DeBari, M.N. Keyser, M.A. Bai, R.D. Abbott, 3D printing with silk: considerations and applications, Connect. Tissue Res. 61 (2) (Mar 2020) 163–173, https://doi.org/10.1080/03008207.2018.1553959.
[183] H. Qiao, K. Zheng, Three-dimensional printing to build fibrous protein architectures, Methods Mol. Biol. 2347 (2021) 177–189, https://doi.org/10.1007/978-1-0716-1574-4_16.
[184] K. Schacht, T. Jüngst, M. Schweinlin, A. Ewald, J. Groll, T. Scheibel, Biofabrication of cell-loaded 3D spider silk constructs, Angew. Chem. Int. Ed. Engl. 54 (9) (Feb 23, 2015) 2816–2820, https://doi.org/10.1002/anie.201409846.
[185] A. Lechner, V.T. Trossmann, T. Scheibel, Impact of cell loading of recombinant spider silk based bioinks on gelation and printability, Macromol. Biosci. 22 (3) (Mar 2022) e2100390, https://doi.org/10.1002/mabi.202100390.
[186] E. DeSimone, K. Schacht, A. Pellert, T. Scheibel, Recombinant spider silk-based bioinks, Biofabrication 14 (9) (2017) 044104, https://doi.org/10.1088/1758-5090/aa90db.
[187] J.W. Kuhbier, C. Allmeling, K. Reimers, A. Hillmer, C. Kasper, B. Menger, G. Brandes, M. Guggenheim, P.M. Vogt, Interactions between spider silk and cells—NIH/3T3 fibroblasts seeded on miniature weaving frames, PLoS One 9 (5) (2010) e12032, https://doi.org/10.1371/journal.pone.0012032.
[188] J.W. Kuhbier, K. Reimers, C. Kasper, C. Allmeling, A. Hillmer, B. Menger, P. M. Vogt, C. Radtke, First investigation of spider silk as a braided microsurgical suture, J Biomed Mater Res B Appl Biomater 97 (2011) 381–387, https://doi.org/10.1002/jbm.b.31825.
[189] K. Hennecke, J. Redeker, J.W. Kuhbier, S. Strauss, C. Allmeling, C. Kasper, K. Reimers, P.M. Vogt, Bundles of spider silk, braided into sutures, resist basic cyclic tests: potential use for flexor tendon repair, PLoS One 17 (8) (2013) e61100, https://doi.org/10.1371/journal.pone.0061100.
[190] C. Qing, Q.Y. Li, N.N. Xue, S.M. Yuan, C.J. Liu, C.G. Zhang, H.W. Li, Y. Zhao, The outlook of the development of innovative products from biocompatible natural spider silk in the beauty thread-lifting industry, Nat. Prod. Bioprospect. 11 (2021) 21–30, https://doi.org/10.1007/s13659-020-00291-9.
[191] S.L. Adrianos, F. Teulé, M.B. Hinman, J.A. Jones, W.S. Weber, J.L. Yarger, R. V. Lewis, Nephila clavipes flagelliform silk-like GGX motifs contribute to extensibility and spacer motifs contribute to strength in synthetic spider silk fibers, Biomacromolecules 14 (6) (Jun 10, 2013) 1751–1760, https://doi.org/10.1021/bm400125w.
[192] A.T. Sensenig, S.P. Kelly, K.A. Lorentz, B. Lesher, T.A. Blackledge, Mechanical performance of spider orb webs is tuned for high-speed prey, J. Exp. Biol. 216 (Pt 18) (Sep 15, 2013) 3388–3394, https://doi.org/10.1242/jeb.085571.
[193] X. Qiao, Z. Qian, J. Li, H. Sun, Y. Han, X. Xia, J. Zhou, C. Wang, Y. Wang, C. Wang, Correction to synthetic engineering of spider silk fiber as implantable optical waveguides for low-loss light guiding, ACS Appl. Mater. Interfaces 10 (42) (Oct 24, 2018) 36582, https://doi.org/10.1021/acsami.8b14593.
[194] M. Reimer, K. Mayer, D. Van Opdenbosch, T. Scheibel, C. Zollfrank, Biocompatible optical fibers made of regenerated cellulose and recombinant cellulose-binding spider silk, Biomimetics (Basel) 8 (1) (Jan 15, 2023) 37, https://doi.org/10.3390/biomimetics8010037.
[195] F.G. Omenetto, D.L. Kaplan, New opportunities for an ancient material, Science 329 (5991) (2010) 528–531.
[196] J.F. Buyel, Plant molecular farming - integration and exploitation of side streams to achieve sustainable biomanufacturing, Front. Plant Sci. 2019 (1893) 9.
[197] G. Bhattacharyya, P. Oliveira, S.T. Krishnaji, D. Chen, M. Hinman, B. Bell, T. I. Harris, A. Ghazitabatabaei, R.V. Lewis, J.A. Jones, Large scale production of synthetic spider silk proteins in *Escherichia* coli, Protein Expr. Purif. 183 (2021) 105839.
[198] J. Li, S. Li, J. Huang, A.Q. Khan, B. An, X. Zhou, Z. Liu, M. Zhu, Spider silk-inspired artificial fibers, Adv. Sci. (Weinh.) 9 (5) (Feb 2022) e2103965, https://doi.org/10.1002/advs.202103965.
[199] L. Ye, X. Liu, K. Li, X. Li, J. Zhu, S. Yang, L. Xu, M. Yang, Y. Yan, J. Yan, A bioinspired synthetic fused protein adhesive from barnacle cement and spider dragline for potential biomedical materials, Int. J. Biol. Macromol. 253 (Pt 5) (Dec 31, 2023) 127125, https://doi.org/10.1016/j.ijbiomac.2023.127125.
[200] E. DeSimone, K. Schacht, A. Pellert, T. Scheibel, Recombinant spider silk-based bioinks, Biofabrication 9 (4) (Nov 14, 2017) 044104, https://doi.org/10.1088/1758-5090/aa90db.
[201] A. Lechner, V.T. Trossmann, T. Scheibel, Impact of cell loading of recombinant spider silk based bioinks on gelation and printability, Macromol. Biosci. 22 (3) (Mar 2022) e2100390, https://doi.org/10.1002/mabi.202100390.
[202] V.T. Trossmann, S. Heltmann-Meyer, H. Amouei, H. Wajant, R.E. Horch, D. Steiner, T. Scheibel, Recombinant spider silk bioinks for continuous protein release by encapsulated producer cells, Biomacromolecules 23 (10) (Oct 10, 2022) 4427–4437, https://doi.org/10.1021/acs.biomac.2c00971.