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Review

A review on advances in the applications of spider silk in biomedical issues

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ABSTRACT

Spider silk, as one of the hardest natural and biocompatible substances with extraordinary strength and flexibility, have become an ideal option in various areas of science and have made their path onto the biomedical industry. Despite its growing popularity, the difficulties in the extraction of silks from spiders and farming them have made it unaffordable and almost impossible for industrial scale. Biotechnology helped production of spider silks recombinantly in different hosts and obtaining diverse morphologies out of them based on different processing and assembly procedures. Herein, the characteristics of these morphologies and their advantages and disadvantages are summarized. A detailed view about applications of recombinant silks in skin regeneration and cartilage, tendon, bone, teeth, cardiovascular, and neural tissues engineering are brought out, where there is a need for strong scaffolds to support cell growth. Likewise, spider silk proteins have applications as conduit constructs, medical sutures, and 3D printer bioinks. Other characteristics of spider silks, such as low immunogenicity, hydrophobicity, homogeneity, and adjustability, have attracted much attention in drug and gene delivery. Finally, the challenges and obstacles ahead for industrializing the production of spider silk proteins in sufficient quantities in biomedicine, along with solutions to overcome these barriers, are discussed.

1. Introduction to spider silk

More than 40,000 species of spiders have been identified, and it is estimated that there are as many or even more spider species that are not yet known. All types of true spiders spin at least one type of silk and sometimes more depending on their glands [1]. The silks have different characteristics depending on their applications, including making the main strands of the nest, the connection radius of the main strands, or the prey hunting [2]. Spider silk, known as one of the hardest natural substances made by living organisms, is made up of proteins [3,4], and due to the nature of its ingredients, it is a biocompatible and environmentally friendly material [4]. Other unique features of spider silk are outstanding mechanical properties, excellent fatigue behavior, high energy absorption, biocompatibility, and low biodegradation rate [5–7]. Different types of spider silk fibers can have different properties, such as low density, high expandability, and high tensile strength [6]. For example, MaSp1 fibers have high tensile strength, and flagelliform silks have high expandability (Table 1). These salient features have attracted the attention of researchers for industrial and medical use [4,7]. Materials derived from spider silk, besides the adjustability of the rate of their biodegradation can retain their stability and mechanical properties in biological environments for a long time [8]. By-products of spider silk are non-toxic, low in immunogenicity, and remove easily from the body [7,9].

Orb-web weaving spiders have seven glands in their abdomen, including the minor ampullate, the major ampullate, the aggregate, the flagelliform, the pyriform, the aciniform, and the cylindriform glands that produce and secrete silks in liquid form, which immediately solidify after secretion (Fig. 1). The auxiliary spiral fiber is secreted from the minor ampullate gland, while the major ampullate gland secretes dragline silk that is very strong and forms the main strands of the nest. The aggregate gland secretes fibers to create a sticky aqueous coating. The flagelliform gland secretes fibers to form the core of the capture spiral. The pyriform gland secretes fibers as cements for joints and attachments, and the aciniform gland secretes fibers for swathing prey and creating the soft inner silk of the egg sack. Finally, the cylindriform

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gland secretes fibers to form rigid external silk of the egg sack [10].

Spider silks are mainly composed of glycine and alanine amino acids [11,12]. Spider silk proteins are relatively heavy and large. For example, for the MaSp1 type of spider silk, a molecular weight of about 300 kDa has also been reported [13]. The fibers are composed of a non-repetitive amino-terminal space domain, a broad central domain of spider silk proteins, and a non-repetitive carboxyl-terminal space domain [14,15]. The main domain consists of 100 repeats of the glycine and polyalanine amino acid sequences, accounting for approximately 60-90% of the fibers [16]. The degree of crystallinity, which determines the strength and stiffness of the fibers are determined by the number of alanine blocks in the main domain. On the other hand, Glycine residues play an important role in the expandability and resilience of the fibers [17].

The crystal part of spider silk, which is made up of highly durable layered β-sheets and also the fine structure consisting of uniaxially oriented protein molecules [18], provides superb tensile strength [4,19]. Tensile strength depends on the molecular interactions of hydrogen bonds. The thermal stability, modulus, and tensile strength of the sheets improve as the number of hydrogen bonds in the β-sheets increases [4,20].

Spider draglines are suitable for estimating actual mechanical stress because the total weight of the spider is applied when it falls on the dragline. One study also described the real stress caused by the spider's weight on the *Nephila clavata* spider draglines when the spider fell [21]. Dragline silk is used as a framework thread for the spider web, and spiders use it as a guide to return to the starting spot, as a safety line (or lifeline) for unexpected falls, for locomotion and chemical communication. Dragline silk holds extraordinary mechanical properties (modulus, tensile strength, and the toughness of 10 GPa, 1.652 GPa, and 354 MJ/m^3, respectively) is the subject of more than 90% of researches on spider silk [3,4,6,22,23].

Osaki et al. examined the elastic modulus of curves of draglines secreted by *Nephila clavata* spiders at different stages of their development, with an elastic modulus of about ca. 10 GPa for spiders weighing less than 50 mg, ca. 13 GPa for spiders between 50 and 800 mg, and ca. 10 GPa was determined for older spiders weighing more than 800 mg [24]. Different mechanical properties of dragline silks from different species of spiders, recombinant spider silk fibers, as well as Kevlar and Steel (two artificial materials with outstanding mechanical properties) are presented in Table 1.

The production of spider silk is affected by varying environmental conditions [19]; for instance, a reversible phenomenon known as supercontraction occurs in high humidity. The supercontraction refers to softening and reducing the length of spider silk up to 60%, resulting in the transition from a heavily oriented glass phase to an irregular rubber phase [25]. The features of supercontraction are determined by specific motifs in silk proteins [19], the spider species [25], the spinning procedure [3], and the amount of proline [6].

Despite having many advantages and remarkable characteristics, spider silk fibers were not used in early researches for medical applications because they were difficult to obtain in large quantities from natural sources. However, courtesy of the recent advances in molecular biology techniques, researchers have recently become interested in producing spider silk fibers using recombinant DNA technology [7,26,27]. The differences in codon usage between spiders and the host organisms affect recombinant silk processing. Furthermore, bacterial hosts often delete repetitive sequences via the homologues recombination process [7]. Biotechnological processing can also alter the properties of spider silk proteins chemically and genetically [14,28].

2. Different forms of spider silk

Spider silk proteins can develop into different morphologies based on their processing and assembly procedures [28,29]. Spinning dope, which is a highly concentrated solution containing spider silk protein, can be used to convert spider silk into various forms. This spinning dope is created by dissolving the recombinant spider silk or re-solubilizing the natural spider silk. Due to the less availability of natural spider silk, recombinant spider silk is usually used to create spin dopes. The most important morphologies are fiber, foam, film, hydrogel, non-woven, spheres, and capsules. The more explicated data on these morphologies have been brought in Table 2, and a summary in which these morphologies are developed has been presented in Fig. 2.

2.1. Fiber

Fiber, as a one-dimensional assembly of spider silk proteins with enriched β-sheet content, is applied in biomedical applications [7], developing protective tools and composites [7,30] and violin strings [31]. Fiber assembly in the spinning duct requires physical and chemical changes in proteins leading to the phase transition of liquid-solid, followed by elongation and mechanical shearing [29]. The toughness of spider silk fibers depends on parameters such as tensile strength, extensibility, and density. The amount and type of combination of these parameters define the toughness of silk. Usually, in the production of recombinant spider silk, attention is paid to improving properties such as tensile strength, which reduces the amount of fiber strain and thus makes it brittle [32]. Since the biocompatibility of the spider silk fibers, there have been so many attempts to generate fiber-based scaffolds and medical sutures [33]. For instance, manufacturing the scaffolds for human neurons [7], tendons replacement [30], and skin repair in plastic surgery [7] have been reported. There are different approaches to spin

Table 1
Mechanical properties of dragline silks derived from spiders in comparison to some other materials.

Material	Tensile strength (MPa)	Extensibility (%)	Young's modulus (GPa)	Toughness (MJ·m^{-3})	References
Natural *N. clavipes* dragline spidroin	1215 ± 233	17.2 ± 3.5	13.8 ± 3.6	111.2 ± 30	[140]
Natural *A. diadematus* major ampullate silk	1100	27	10	160	[141]
Natural *N. edulis* dragline spidroin	1300 ± 100	39 ± 6	10.5 ± 1.2	–	[142]
Natural *A. Gemmoides* cocoon silk	230	19	7	70	[143]
Natural *A. trifsaciata* aciniform spidroin (AcSp1)	687 ± 56	86 ± 3	–	376 ± 39	[144]
Natural flagelliform silk (*A. diadematus*, *A. sericatus*, A, argentata)	500-1300	119-270	–	75-283	[145]
Recombinant AcSp1	115.06 ± 24.44	37 ± 11	–	33.83 ± 13.45	[146]
Recombinant *A. diadematus* ADF3	64.6 ± 26.0	10.8 ± 3.1	–	–	[147]
Recombinant *N. clavipes* MaSp1	508 ± 108	15 ± 5	–	–	[148]
Recombinant *A. ventricosus* flagelliform spidroin	41.5-107.54	2.73-58.76	2.42-4.89	0.49-19.46	[149]
Bombyx mori cocoon silk	600	18	7	70	[150]
Bone	160	3	20	4	[151]
Collagen	150	12	1500	7.5	[151]
Kevlar 49	3600	2.7	130	50	[152]
High-tensile steel	1500	0.8	200	6	[153]

the recombinant spinning dope into fibers, like wet spinning [34] and spinning through microfluidic devices [35] and electrospinning. One of the most common and easy methods of forming fiber is electrospinning, in which a variety of organic and aqueous solvents are used to create spinning dope [36]. An aqueous electrospinning dope has more difficult preparation steps but at the same time improves the safety and performance of the resulting fiber. After preparing aqueous electrospinning dope, the post-treatment step is performed, which has different types and purposes, for example, to induce the formation of β-sheet crystal and lead the proteins to become insoluble in water and form fibers. During the electrospinning process, by passing and flowing aqueous electrospinning dope through a needle with a specific diameter and length, sufficient voltage is applied to it in an electrical field, and the resulting fiber is collected on the collector. Various parameters such as solvent properties and concentration, set-ups, voltage, and environmental conditions such as temperature and humidity are involved in this process and the quality of the resulting fiber [37]. Many attempts have been made to produce recombinant spider silk fiber. According to the various factors involved in the production process, different results have been obtained for the mechanical properties, listed in Table 2.

2.2. Film

Film is a two-dimensional assembly of spider silk proteins obtained through evaporation of organic solvents or aqueous solutions [7,28,29]. It has been demonstrated that as the β-sheet content of films increases, a rise in strength, stiffness, and fragility of material is seen while the elasticity of the material decreases [7]. The advantage of using film over fiber is that it is created only by pouring a certain amount of spinning dope into a container with the desired diameter and drying it, and there is no need to spend spinning costs [38].

There are many methods to produce a film of spider silk protein solution. In one of these methods, the protein solution containing plasticizer is centrifuged and filtered, then its concentration is measured by the NanoDrop method. Next, the spider silk protein solution is diluted with a buffer, poured on polytetrafluoroethylene cards according to the desired diameter, and dried in a laminar flow cabinet [39]. In another method, a spin-coater device is used, in which a solution of spider silk protein is placed in the center of the substrate and spin-coated. The protein solution is cast on silicon wafers and dried overnight or in a desiccator to form a film [40].

Spider silk films are robust and comfortable tools mainly to detect and characterize recombinant proteins [41]. Due to films have no toxicity and side effect inside the body, they are used as implant coatings and tissue engineering scaffolds [29,42]. Also, applications of spider silk films are expanded to the fields of cell response screening against biochemical features [41], drug delivery [29,43], skin and tendons replacement [30], and biochemical sensors [29]. In a study on engineered recombinant ADF4 spider silk, various aqueous and organic solvents were used to investigate the resulting film's mechanical properties and thermal stability. The solvents used in this study were formic acid (FA), hexafluoro-2-propanol (HFIP), and aqueous solvents. The results showed that thermal stability did not change much. Still, the mechanical properties in different solvents and post-treatment with methanol showed changes. The highest tensile strength was obtained by dissolving in FA and treatment with methanol [44]. In another study on the thermal conductivity capacity of spider silk films produced in HFIP solvent, it was shown that increasing a-helices in the film structure

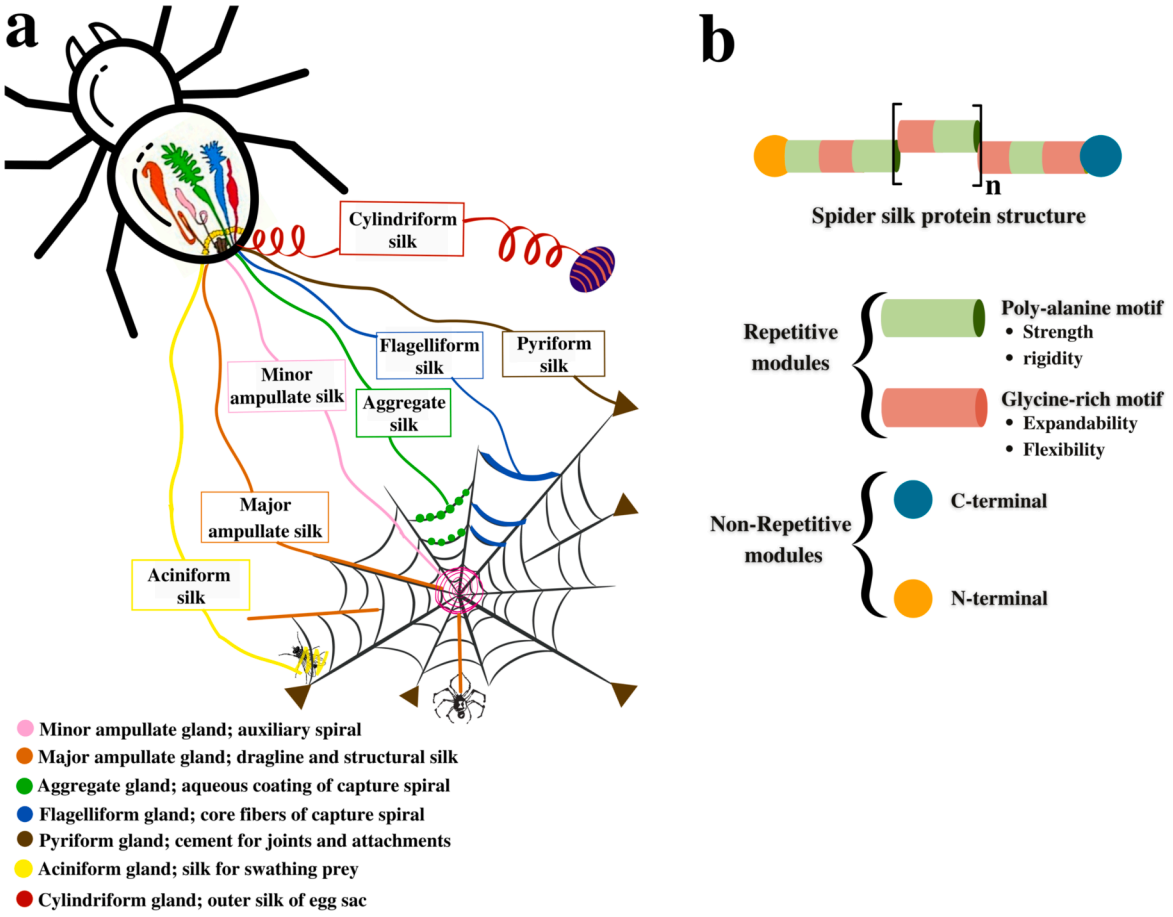

Fig. 1. (a) Seven types of silk glands in a female spider and the silk produced by each gland (b) primary structure of the spidroin including a repeating core domain and non-repeating N and C terminals [2].

Table 2
The properties of some spider silk morphologies.

Morphology	Structure	Applications	Thermal conductivity (W/m·K)	Young modulus (MPa)	Toughness (MJ/m^3)	Strength (MPa)	Break strength (%)	Reference
Fiber	1D	Biomedical sutures, tissue engineering	416 (*Nephila clavipes* dragline silk)	As-spun: 2780 ± 530 Stretched: 5700 ± 2430	As-spun: 0.87 ± 0.63 Stretched: 23.73 ± 18.46	As-spun: 35.65 ± 8.42 Stretched: 132.53 ± 49.20	As-spun: 3.13 ± 1.84 Stretched: 22.78 ± 19.06	[154–158]
Film	2D	Implant coating, screening the cell response against biochemical features, drug release, tissue engineering, biochemical sensors	348.7 ± 33.4 to 415.9 ± 33.0	Methanol treated: 3500 ± 300 As Cast: 2900 ± 400	Methanol treated: 790 ± 80 As Cast: 770 ± 190	Methanol treated: 71 ± 2 As Cast: 60 ± 5	Methanol treated: 2.2 ± 0.1 As Cast: 2.5 ± 0.4	[154,158–162]
Non-woven	2D	Face masks, air filtrating systems, wound healing, tissue engineering	–	~1000	~5	~60	~10	[163–165]
Foam	3D	Tissue engineering, reduction of internal bleeding	–	0.001	–	–	–	[154,155,166]
Hydrogel	3D	Tissue engineering, bio-inks in 3D bioprinting, drug delivery	–	0.0084	–	–	–	[155,160,167,168]
Sphere	3D	Encapsulating substances, drug delivery	–	–	–	–	–	[156,169]
Capsule	3D	Encapsulating molecules, drug delivery, protective container for enzymatic reactions, microreactor design, filtration of micro- and macro-molecules	–	700–3600	–	–	–	[156,159,170–173]

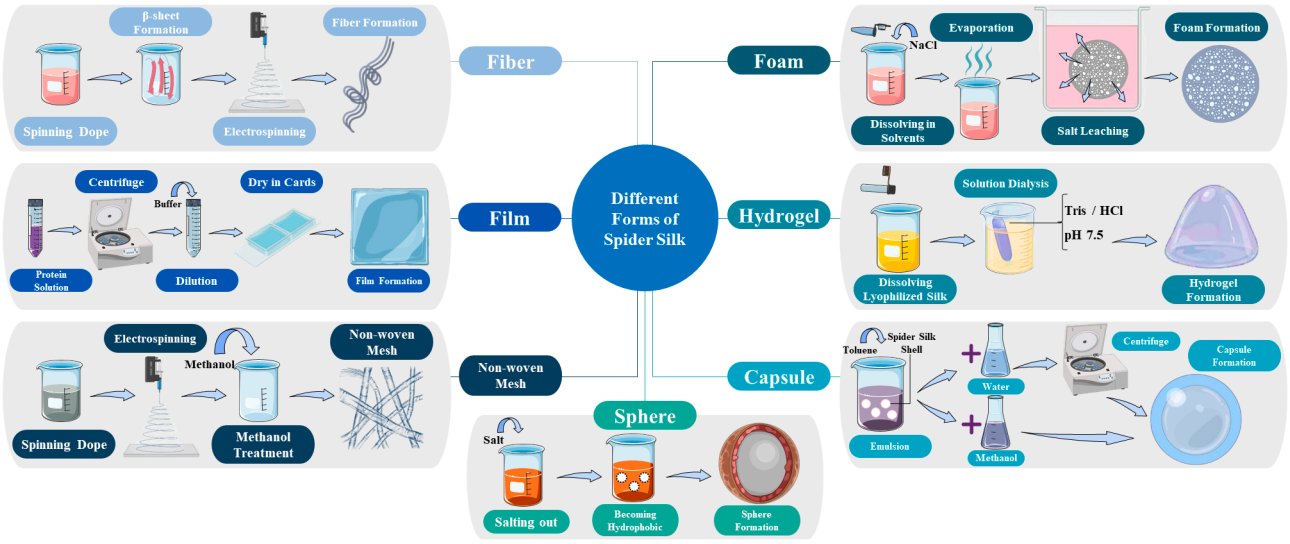

Fig. 2. Different forms of spider silk are used in different applications, which are discussed in this article. This figure briefly outlines the preparation process of the various forms of spider silk and their final morphology. The spider silk proteins used in these methods are of natural origin or synthesized by recombinant methods.

increases the thermal conductivity [45].

2.3. Non-woven mesh

Scientists suggest that non-woven materials have characterizations of both two-dimensional films and three-dimensional networks. In the production of non-woven meshes, centrifugal electrospinning is confirmed to be the most efficient and cost-effective technique [46,47]. After the fiber is spun during the electrospinning process, it randomly precipitates on the counter electrode to form a nonwoven mesh. Methanol treatment then stabilizes the properties of the fibers by inducing β-sheet structures. Different concentrations of protein solutions lead to the production of fibers with different diameters and pore sizes in the nonwoven mesh [48]. Meshes have random or aligned fiber orientations and are equipped with knotting, twisting, and many interconnections [7,49].

Along with excellent air permeability, non-woven meshes are applied in designing filtration systems (nanofibrous or sub-micron fibrous) [49]. As non-woven meshes mimic structural features of extracellular matrix (ECM), they are applied to chemical air filtrating systems and face masks, tissue engineering and wound healing applications [50]. One study investigated the mechanical properties of irregular fiber bundles at different relative humidity under as spun and post-treated with ethanol conditions. Almost all of these properties were shown to be improved post-treated with ethanol condition. Data on the mechanical properties of irregular fiber bundles at 30% relative humidity are shown in Table 2 [51].

2.4. Foam

As a three-dimensional structure, foam contains numerous small bubbles obtained from spider silk proteins [47]. Foams are formed through salt leaching, gas foaming, and freeze-drying processes [52]. Since foams have mechanical stability and good transportation of

nutrients and wastes, scientists have suggested using them as scaffolds in cell cultures and tissue engineering [7,53]. Also, foams have been applied for reducing internal bleeding in trauma victims [30]. To produce foam from spider silk protein by salt leaching method, it is first dissolved in solvents. Then ground and sieved NaCl is added to the protein solution as porogen with specific weight percentages and ratios. The solvent is then evaporated for at least 48 h, and then the salt is washed with milli-Q water for 24 h with at least six water changes. For storage, it can be stored in milli-Q water at room temperature or lyophilized. The size of foam pores is adjusted by salt crystal size, and its mechanical properties are adjusted by protein concentration [54].

2.5. Hydrogel

Hydrogel, the most physiologically exact morphology obtained from spider silk, is a three-dimensional network formed via the connection of proteins and polymers in an above 95% water-based solution by either chemical cross-linking or physical cross-linking of polymers [41,55]. Changes in pH, centrifugation, and ultra-sonication can facilitate this process [56]. A biodegradable hydrogel has been demonstrated to be produced by self-assembly based on the density of fibers, especially non-branched fibers. In this thermodynamically-regulated process, temperature and concentration of proteins have critical roles in hydrogel formation since the size of hydrogel pores is determined by protein concentration [41,55]. The pore size of spider silk hydrogels can be easily adjusted by changing the concentration of the protein solution before forming the hydrogel [57]. As this concentration increases, the formation of β-sheet rich nanofibrils and a rise in the elastic and shear modules occur [55]. In the dialysis method for hydrogel formation, the lyophilized spider silk is dissolved in the solvent, and dialysis membranes are used in the presence of Tris/HCl at pH 7.5 for one night at room temperature for solution's dialysis. The gelation process depends on the concentration of the protein solution and does not occur at less than a certain concentration [55]. Some applications of the hydrogels expand to their use in cell cultures, bioinks of 3D bioprinting, drug delivery, and determination of drug release profile [7,41,56]. A problematic issue in hydrogel-based drug delivery is the uncontrollable release of drugs which can be solved using approaches like the production of multimembrane hydrogels [41]. Hydrogel is one of spider silk's forms that are very suitable for use as a bioink and is used to produce 3D cell-loaded constructs. Hydrogels are an excellent option for cell scaffolds because they allow the proper release of oxygen and nutrients and can also move cell excretions around them [58].

2.6. Sphere

Another non-toxic morphology obtained from spider silk, the sphere, is formed through relevant hydropathy of the protein or the salting-out methods [28,59]. Among the various methods used to form the sphere, salting out with potassium phosphate ions in the existence of shear forces caused by blending mimics the natural spinning conditions of spider silk [60]. Through the salting-out process, potassium phosphate is rapidly mixed with the solution of spider silk protein [33,59,61]. The salt concentration leads to an alternation in the interactions between protein and water, and a rise in hydrophobic interactions between silk molecules can be seen [59,62]. Many factors affect the formation of the sphere, including the amino acid sequence and the concentration of spider silk protein, the pH of potassium phosphate, and the concentration of potassium. When using higher ion concentrations, it has been shown that more stability and better formation are seen in the sphere [60]. Like other forms of spider silk, spheres have properties such as high toughness, slow biodegradation as well as being rich in β-sheet structures [28,33]. The most important application of silk spheres is encapsulating drugs in the field of drug delivery [28,33].

2.7. Capsule

Recombinant spidroins have amphiphilic characteristics, thus, assembling water-oil interfaces [28]. In one study, eADF4 (C16) silk capsules were mechanically and chemically very stable and had a high β-sheet content, despite being thin with Young's modulus of 0.7–3.6 GPa [63]. Due to this characteristic, β-sheet rich capsules, which are thin and stable, can be formed by emulsion processes [28,33,64]. A dialyzed protein solution is emulsified in toluene for one and a half minutes to produce spider silk microcapsules. Shells of spider silk then surround the emulsion droplets. To transfer these microcapsules to a single-phase solution, either adding water and creating an aqueous sublayer and then centrifuging is used, or by adding ethanol, the two-phase solution is converted to a single phase. The size of the resulting microcapsules depends on the size of the emulsion droplets so they can be controlled [64]. Also, eADF4 (C16) spider silk capsules without toluene, which is a toxic substance, were produced by emulsion method by replacing silicone oil as the organic phase [63]. These capsules have porous structures which filtrate micro-molecules from macro-molecules [33]. Furthermore, their capacity for encapsulating and protecting almost all kinds of molecules is interesting [14]. Besides, capsules can be utilized as delivery carriers for substances like dyes, drugs and, flavors, in which the release of these substances via proteolysis of the spider silk proteins [33,64,65].

3. Chemical and genetic modifications of spider silk

To develop new or more efficient silks, chemical and genetic modifications are applied biotechnologically to spider silk proteins [41].

3.1. Chemical modifications

Performing chemical modifications on silk proteins on hydroxyl-terminated tyrosine and serine residues, as well as carboxyl-terminated glutamic acid and aspartic acid residues, is possible [28]. Introducing electrophilic and nucleophilic groups to the amino acids of spidroins allows scientists to perform site-specific modifications [66]. The toxicity of some chemical reagents limits the utilization of chemically-modified spidroins in biomedical applications [28].

Coupling reactions, amino acid modifications, and grafting reactions have been suggested as methods for chemical modification. Modifying silks through coupling reaction is determined by side chain hydroxyl- and carboxyl- containing amino acid residues, which is used to immobilize enzymes, drugs, and other substances on the silk protein. In amino acid modification, polar groups of amino acid residues are modified to regulate the hydrophobicity of silk proteins. In grafting reaction, conjugation of protein-polymer occurs, which leads to the significant bioactivity of modified silk proteins [66].

One of the limitations of the copper-catalyzed azide-alkyne cycloaddition coupling (CuAAC) method is the addition of cross-links between spidroin molecules because spidroins have free carboxyl groups. Another limitation is the presence of undesired cross-links between molecules that have both carboxyl and amino groups. To prevent an autoimmune response to covalent aggregates created from cross-linked therapeutic molecules, CuAACs are designed highly accurately to prevent unwanted cross-linking [67].

Silk proteins undergo chemical modifications by modifying existing reactive side chains or introducing a natural reactive group to the protein. One of the limitations of this method is that the treatment of existing reactive side chains seems to be an uncontrollable process and may change the protein's overall structure and negatively affect its other properties [68]. For example, it may change its solubility, cause folding problems, or alter the overall protein surface charge. An example relevant to the first case is the modification of silk glutamic acid residues through their coupling with β-galactosidase on the film surface, followed by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-

hydroxysuccinimide (EDS/NHS)-based activation of glutamic acid residues to be used as biosensors [28]. An example of the second case is introducing cysteine-containing modules to the spider silk protein for developing conductive materials. Cysteine-containing proteins can be modified through coupling with fluorescein-maleimide since having a thiol group [28]. Another important application of this type of modification is in developing antimicrobial materials. In the silk-based textile industry, chemically modified silk dressings are built by adding 3-trimethylsilylptopyl-dimethyloctadecyl ammonium chloride to silk to supplement some antibacterial properties beneficial in the atopic dermatitis cure [43].

3.2. Genetic modifications

Genetic approaches in the modification of spidroins via the production of silk hybrids (i.e., the addition of a functional peptide sequence to silk sequence) have expanded silk technologies to develop drug delivery systems, wound healing tools, scaffolds for tissue engineering purposes, and coating for implants [28,50,53]. The process in which genetic modifications are performed, along with functional motifs which can be conjugated to spider silk protein, and their applications in silk technologies, are shown in Fig. 3.

RGD (Arg-Gly-Asp) is a cell-binding motif that improves the attachment and proliferation of cells in biomaterial applications [40]. In a study, RGDs were added to the poly-L-lysine-spider silk hybrids to promote integrin-mediated endocytosis [43,50]. Interestingly, the fusion of 11 sequences of RGD and poly-L-lysine with spider silk increased cellular uptake efficiency [28,50]. Also, RGD motifs enhanced β-sheet formation in spider silk, retaining the stability of silk films [69]. In addition, it has been found that the fusion of cell penetrating peptides (like ppTG1 peptide) with spider silks promotes gene delivery and allows for gene release control [50]. If the RGD sequence is located near the chain ends,

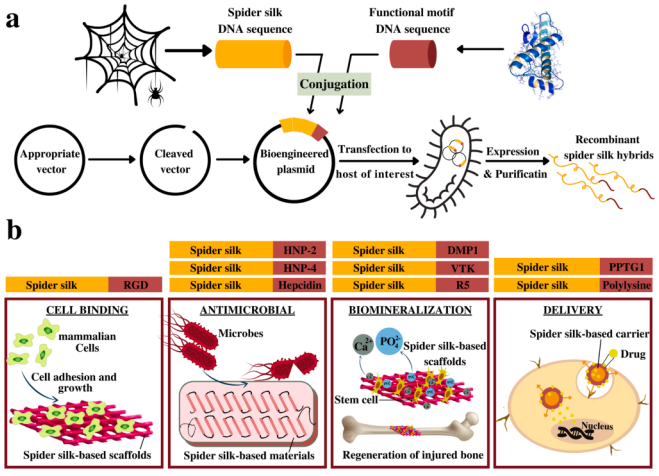

Fig. 3. This illustration aims to provide a summary of the process in which Genetic modifications are performed on spider silks, besides representing some important applications of recombinant spider silk hybrids in biopharmaceutical industry. (a) Genetic modification in which a DNA sequence of a functional motif conjugates to the DNA sequence of spider silk has been shown, along with the production of a recombinant DNA, its transfection to the desired host, and ultimately its expression to a more functional protein. (b) Some functional motifs can be added to the recombinant spider silk protein structure through genetic modifications to make improvements in their properties and functions including their roles in biomineralization, delivery system, cell adhesion, and antimicrobial activity. Schematic designs for each application are provided, and different parts of each are introduced as well. At the top of each part, we have demonstrated the structure of the recombinant spider silk chimeras (yellow block: the name of the effective motif, red block: spider silk amino acid sequence). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

it may, after self-assembly and the formation of β-sheets, be in a place where it is so-called hidden and cannot play its role and cause cell adhesion [69].

According to the evidence, spider silks are also applicable in biomineralization, such that identification of the motifs relevant to the calcification has enabled scientists to design either strong (for bone regeneration) or flexible (for heart valves) biomaterials [70]. In the case of biomineralization, the fusion of R5 peptide [28], dentin matrix acidic phosphoprotein1 (DMP1), and hydroxyapatite binding domain (VTK) [70] with spider silks are evaluated. In a study, researchers fused the repetitive motif of R5 peptide from silaffins of a diatom, *Cylindrotheca fusiformis*, with the C- or N-terminus of the RGD-containing consensus repeat of silk protein [70]. As a result, they observed silica in silk films loaded with these chimeric proteins, which could be applied in biomineralization and bone formation [28]. Recombinant spidroins, based on the fusion of DMP1 with *Nephila clavipes* silk, can lead to hydroxyapatite (HAP) controlled mineralization. When incubating these recombinant proteins with $CaCl_2$, interactions between the Ca^{2+} and HA-nucleating domain of DMP1 lead to the formation of β-sheet and α-helices structures, suggesting a preserved β-sheet formation even after fusion. Films made from these chimeric proteins can play a role in bone regeneration and osteoblastic differentiation in 3D scaffolds [71]. Natural spider silk scaffolds can play a role in preserving material stability, whereas recombinant spider silk proteins functionalized with the VTK domain can also regulate osteogenesis through hydroxyapatite formation [70,72].

Spider silk protein chimeras can also have antimicrobial and bactericidal effects in tissue engineering. In a study, antimicrobial peptides from *Homo sapiens* (hepcidin, human neutrophil protein-2 (HNP-2), human neutrophil protein-4 (HNP-4)) were fused to the spider silks [50]. These chimeras were compatible with mammalian cells. Interestingly, it has been shown that after fusion, β-sheet formation (responsible for the stability of mechanical features) and self-assembly procedure retain in the silks [73].

One of the modifications that are done to a protein is a change in the main block of the polymer, such as the removal, addition, or substitution of some amino acids to improve some of the proteins' properties or add a feature for a specific application. These changes can alter some of the desired properties of the protein or affect its folding. It is possible to predict and track these problems with precision engineering in silico, but it is challenging and may face numerous issues. Due to the incorrect predictions, the motifs may appear in the middle of the β-sheet structure, for example, or prevent its self-assembly and destroy the desired properties of the polymer. Also, the desired motif may not be in the place it should be for it to function due to protein folding.

4. Spider silk in tissue engineering and regenerative medicine

Tissue Engineering is an interdisciplinary field that develops biological substitutes to restore, maintain, and improve tissue function or a whole organ [74,75]. Progress in this area largely depends on biomaterials that can mimic the target ECM traits or induce favorable cell differentiation [76]. Biomaterials used in tissue engineering must have special properties such as biodegradability, biocompatibility, support for cell adhesion, growth, proliferation, and differentiation, along with appropriate mechanical features [77]. Spider silk stands as an eligible biomaterial and can be an ideal choice aimed at tissue engineering and regenerative medicine [72]. Silk fiber-based implants are also one of the most sought-after solutions in recent decades to facilitate treatment [78]. Some applications of spider silks in several types of engineered tissues and implants are presented in Fig. 4 and discussed as follows.

4.1. Skin regeneration and wound dressing

In ancient times, natural spider silk was used to cover wounds and help the healing process [79], and today, much research has been done

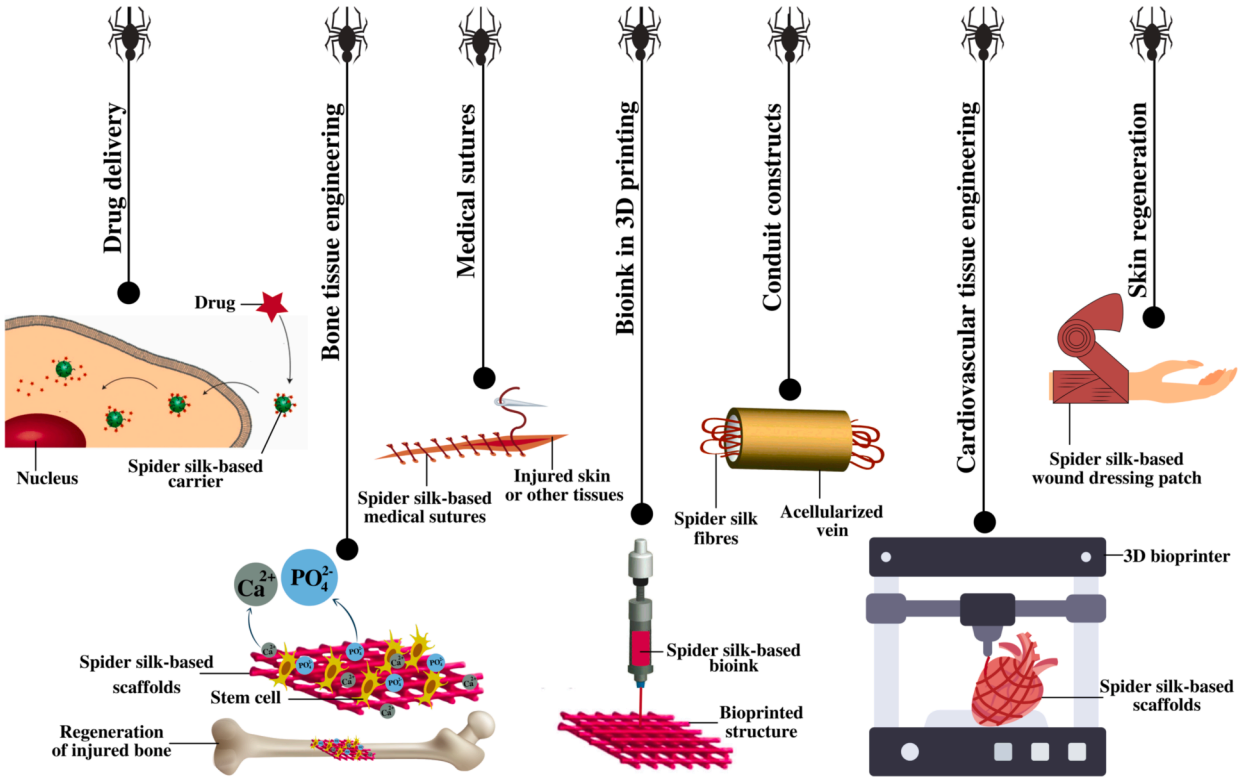

Fig. 4. This figure represents the most important advantages of spider silks, by way of illustration in delivery systems, bone and cardiovascular tissue engineering, conduit constructs, as well as the development of medical sutures, 3D bioprinter bioinks, and wound dressings. Schematic designs for each application are provided, and different parts of each are introduced as well.

on spider silk dressings alone or in combination with other materials such as silkworm silk, which shows its potential to heal burn wounds [79–81]. In a study, spider silk showed antimicrobial properties while supporting the growth of keratinocytes and fibroblasts and was destroyed within eight weeks, so removing the dressing can be omitted from the procedure [80]. In another study, recombinant spider silks attached to RGD-tags were used as wound dressings in second-degree burns of rats. The outcomes demonstrated that spider silks caused suitable expression of basic fibroblast growth factors and synthesis of hydroxyproline, the major amino acid in collagen fibers, which indicated good regeneration in wounded skin [79]. Among the various spider silk morphologies examined to enhance skin regeneration, nonwoven nanofiber matrices appear to be the best type for skin scaffolds [78].

4.2. Cartilage and tendon tissue engineering

Spider silk is a suitable choice as a three-dimensional scaffold in bone, tendon, and cartilage tissue engineering due to its elastic properties and strength among synthetic and natural polymers [82].

Primary chondrocytes of articular cartilage have been discovered to stay attached and live for more than a few weeks once cultured on spider silks [83]. In another study, the growth of chondrocyte cells on spider cocoon silk led to articular cartilage development [53].

It has been shown that micropatterns can facilitate signal transduction and thus differentiation by providing physical and mechanical induction on MSCs without the need for growth factor TGF-β in the culture medium involved in differentiation. In a recent study, the spider silk derived from *Argiope appensa* was used as a bioink to build a micropattern to support cell adhesion and differentiate Human Wharton's jelly MSCs (HWJ-MSCs) to chondrocytes. As a result of this study, it was found that spider silk micropatterns are not only non-toxic and help cells survive but also increase the expression of differentiation markers. The expression level of these markers, such as glycosaminoglycan (GAG) and collagen type II, depends on the concentration of spidroin and the diameter of the fiber used in the micropattern [84].

In another study, a genetically engineered sequence was designed that encoded spider silk-elastin fusion proteins. Transgenic tobacco and potato plants were used as hosts to produce these proteins. Then they used spider silk-elastin fusion proteins to coat culture plates and study the growth rate of anchorage-dependent CHO-K1 cells and Human chondrocytes HCH-371. It was shown that anchorage-dependent chondrocytes in the spider silk and collagen groups had the same rounded shape morphology and chondrocytic phenotype and showed good proliferation [85].

Similar properties of spider silks in tensile behavior and improved fatigue properties with tendons have shown that braided spider silk can be used to repair tendons [5]. It is essential to choose an appropriate biomaterial for the tendon-bone connections known as enthesis to allow tendon replacements. In a study, spider silk hybridized with tags derived from SIBLING proteins such as osteopontin and sialoprotein was used as a desired biomaterial. These tags lead to mineralization, and improvement of calcium phosphate formation by interacting with collagens. This engineered biomaterial was able to support the formation of a pre-osteoblast cell gradient, and these cells remained on the studied hybrid for up to 21 days, indicating that this biomaterial has the potential to support tendon-to-bone connections [86].

Nowadays, using humanoid robotic hands as a prosthesis is one of the new and unique methods in improving the condition of appendage losses. At the core of these humanoid robotic hands must be a fiber similar to the human tendon in order to simulate the transmission of natural hand power. Different materials have been proposed for this purpose but were rejected due to challenges such as high path friction and conductivity. Electro tendon based on *Nephila pilipes* dragline silk is a suitable option with high hardness and conductivity that can show good flexibility and allow the robotic hand to operate without damage

[87].

4.3. Bone and teeth tissue engineering

Despite remodeling capability, bone healing suffers from nonunion fractures and huge defects. Therefore, bone tissue engineering with osteoconductive agents, biocompatible materials, and appropriate cell types is under focus [76,88]. Biomineralization is used to make similar biological materials and has a crucial role in constructing hard tissues like bone and teeth [92]. Direct contact of osteoblasts and dental pulp stem cells with spider silk protein suggested this biomaterial for bone and tooth tissue engineering [89]. The β-sheet structures of the spider silk strengthen the scaffold, and the HAP binding domain (VTKHLNQISQSY or VTK) causes biomineralization and induces calcification. In a study, a genetically engineered spider silk with VTK domain was produced. Human mesenchymal stem cells (hMSCs) were cultured on the mentioned scaffold. Supporting cell proliferation and growth, the addition of the VTK domain to both spider silk terminals increased the formation of crystalline HAP and induced osteogenic differentiation in hMSCs [72]. In another related study, spider dragline silk was used as an organic model for bone mineral HAP nucleation from the supersaturated solution with HAP due to its semi-crystalline structure and repetitive sequences. Spider silk developed crystals of HAP with a favored orientation comparable to natural bone.

A study has shown that eADF4 (C16) with the repetitive core designed by 3D printing exhibits properties that make osteoblast cells offer better adhesion to it [90]. Bone sialoprotein (BSP) is capable of inducing cell differentiation and deposition of calcium phosphates [91]. The AFM results proved that a chimeric protein including spider silk and BSP, due to glutamic acid residues, showed more stiffness than the control groups and in the presence of Ca^{2+} ion and due to high affinity of BSP to this ion, formed supramolecular networks, which promises that this polymer can be used as organic glue in nanocomposite systems for bone tissue engineering [92]. Also, it has been proven that combining these two proteins in a single protein chain does not interfere with the functions of either of them, so that spider silk is able to form the β-sheets, and BSP can induce the nucleation of calcium phosphates [91].

DMP1, in addition to being a dentin protein, is also expressed in various cells that make up hard tissue, such as bone cells. The C-terminal domain of DMP1 has HA nucleating ability. Due to the remarkable mechanical characteristics of spider silk combined with the ability of HA nucleating, this chimeric structure is a major step in the production of composites as well as dental and bone implants [93].

4.4. Cardiovascular tissue engineering

Cardiac tissue engineering and myocardial tissue recovery are applied for treating cardiovascular diseases [94,95]. One of the problems associated with cardiac tissue engineering is the weak beating of the engineered tissue compared with the mature human heart [96,97]; this may be due to cells not reaching the phenotype as adults and their inability to form thick cardiac tissue [98]. In a study, spider silk with a C-terminal attached to the RGD-tag was used. Cardiomyocytes, endothelial, and fibroblast cells were shown to bind properly to spider silk-RGD. As a result, cardiomyocytes responded well to proliferation-induced stimuli, made thick layers, and had faster contractions than the cardiomyocyte monolayers on fibronectin (positive control group) [98].

Another cause of cardiovascular diseases is improper vascular function or its dysfunction, so the replacement of blood vessels can be a successful medication for these types of diseases. In one study, a small-diameter vascular hybrid made of recombinant spider silk/PCL/gelatin was produced using the electrospinning technique. It was implanted intramuscularly in rats for in vivo studies. This research showed that the scaffold made of spider silk/PCL/gelatin presented excellent hemocompatibility and also facilitated the growth of host cells; on the other hand, it did not trigger any genotoxicity and didn't induce any inflammatory factor releases, so it proved to be a decent applicant for small diameter vascular tissues [99].

4.5. Neural tissue engineering and conduit constructs

Traumatic nerve damages have always been one of the most common neurological diseases which cause many irreversible consequences. Due to limited access to autologous transplants, alternative methods such as analogous treatments and tissue engineering have been being studied extensively [100–102]. Extensive nerve gaps can be repaired by creating a suitable environment and conduits for axon growth by engineered structures. The main feature of the design and fabrication of artificial conduits surely is the creation of a frame to cause axonal outgrowth [103]. Spider silk proteins degrade very slowly in vivo with various enzymes, thus allowing the implanted cells to grow and repair the target tissue, and then are removed. One study found that the injured limbs of animals in which spider silk conduits were implanted responded to external electrical stimulation similar to those in isogenic nerve grafts and had the same axon densities, which were myelinated at the distal location of the conduits [104]. Researchers used conduits made of isogenic veins plus spider silks; the sciatic nerve regenerated well in the study groups, proving that spider silk fibers were successful in nerve regeneration [105]. In one study, composites were prepared as an intrinsic framework based on spider silks, acellularized xenogenic veins, and also human stem cells mixed with Matrigel as artificial nerve grafts. The cells adhered quickly and firmly to the spider silks, forming a typical bipolar shape besides columns along the spider silk [106].

Recombinant spider silk was also used as a cell culture matrix to study neuronal growth compared to polylysine. It was shown that the silk films supported cell growth, axonal proliferation, and network connectivity, and neurons grown on them showed amplified expression of neural cell adhesion molecule [107]. Besides, it showed suitable substrate stiffness that modulated axon growth [108]. These results suggest that the physical and chemical properties of spider silk seem quite appropriate for its applications in the treatment of peripheral nerve damages with nerve allografts as endoneurial structural analogs.

5. Bioink in 3D printing

Biofabrication using 3D printing is one of the newest methods in making porous scaffolds of tissue engineering to produce tissues or organs, specifically in the field of personalized medicine [109]. One of the challenges of this method is choosing the right bioink with biocompatibility, appropriate physicochemical properties, high shear thinning behavior, printability, and non-toxicity [58,109]. Having all the mentioned features, spider silks are an ideal choice as a bioink material; moreover, due to their crystallinity, spider silks degrade slowly in bioprinted constructs [110]. The best form of spider silks for being used as bioink is hydrogel. A study has shown that recombinant eADF4 spider silk could be put to use in 3D printing without requiring any cross-linking additives, thickeners, or additional processing [58].

6. Spider silk in delivery of biopharmaceuticals

Drug delivery systems reduce toxic side effects of the drug and frequency of applications while improving cell absorption and bioavailability by reaching an optimum amount of drug in the target location [111]. The drug should penetrate the matrix and not remain just attached to the surface because of possible sudden and rapid release, which is not desirable [112]. The parameters of release rate, loading volume, and loading efficiency determine the suitability of a carrier for the drugs [113]. Various hydrophilic and hydrophobic biomaterials have been used to deliver the drug. One of the disadvantages of hydrophilic materials in the aqueous medium is their dissolution and drug release quickly, and the phenomenon of burst release may occur [114]. Spider silk, which is a hydrophobic biological substance, has all the

desirable properties for drug release [115]. The factors such as pH, type, and concentration of ions are also effective in the characteristics of synthetic spider silk for drug delivery [112,116]. The recombinant spider silk is homogeneous, and its production is easy and adjustable; and these features make recombinant spider silk a suitable delivery agent or diagnostic tool in the biopharmaceutical industry [50].

Among the various forms of recombinant spider silk, the sphere morphologies (mostly eADF4 (c16), MaSp1, and MaSp2 proteins) are more desirable for biopharmaceutical delivery. The eADF4 (c16) protein produces colloidally stable particles and has negative charges at physiological pH. One of the properties of eADF4 (c16) is the slow degradation rate, which results in the controlled secretion of drugs. Lammel et al. showed that submicroparticles of this protein could carry small positively charged molecules properly [113]. In addition, macromolecules such as proteins could also get loaded in eADF4 (c16) with good efficiency [112]. Also, by displacement of Glu with Lys in eADF4 (c16), a protein with a positive charge (called eADF4 (κ16)) was obtained, which was suitable for the delivery of negatively charged drugs [117]. The microcapsules of the mentioned silk were eligible to protect enzymes and sensitive drugs [63,112]. It was shown that the recombinant MaSp1 protein, which was conjugated with peptides named H2.1 and H2.2, could bind precisely to the Her2 receptors with high affinity and was very effective in targeting Her2-positive breast cancer cells [118]. The MaSp2 protein, which has a higher β-sheet content and negative charge due to the difference in its amino acid sequence and formation of smaller spheres, was a suitable carrier for mitoxantrone transfer, while MaSp1 was a more fit option for doxorubicin transfer [119]. Spheres composed of a hybrid protein of MaSp1 and MaSp2 with H2.1 peptide showed better physical and chemical properties than MaSp1 alone with H2.1 while retaining the targeting potential [120].

Recombinant spider silk polymers can be a suitable option for gene delivery due to their unique properties and the ability to create coatings that protect their contents against DNases. Spider silk vectors can be well digested inside the cells in lysosomes, thus release their carrying genes [121]. Engineered spider silk proteins can also be used in order to deliver vaccines, with low unspecific autoimmune toxicity and proper cytotoxic T-cell activation. Most current vaccination strategies cause humoral immunity and usually do not significantly activate cytotoxic T-cells [122]; therefore, cellular immunity is challenging for the vaccine industry [123]. Peptide vaccines alone are not very successful due to rapid degradation and tolerance, which could be overcome with carriers [124]. Since the desired peptide sequence can be added to the silk sequence, spider silks are suitable carriers for peptide vaccines [125]. It has been shown that eADF4 (C16) proteins fused to a cathepsin cleavable peptide as a linker to the antigenic peptide from ovalbumin (as a model peptide antigen) without any immunotoxicity or unspecific immunostimulatory activity could cause a proper antigen-specific proliferation of cytotoxic T-cells in vivo even in the absence of adjuvants. These particles could also be stored for more than a year and sterilized by heat due to their high stability [123]. Applications of spider silks in developing and delivery of biopharmaceuticals are presented in Fig. 4.

7. Spider silk as a suture

The desired suture must have high tensile strength, antimicrobial properties, and biocompatibility to connect and hold the edges of the tissues together at the site of injury, which can be healed without causing any wound infection [126]. In a study to treat flexor tendon injuries, scientists used a spider silk suture and showed that due to the super contraction features of spider silk and the natural environment of a tendon, both materials worked well in water conditions [5].

Axon growth may be prevented by reaction with nylon and polyglycolic acid in micro-sutures, and neuroma formation can occur. In a study, spider dragline silk was produced by braiding it to produce better micro-sutures than nylon sutures, which played an important role in nerve regeneration [127].

Today, various properties, including antimicrobial one, can be added to spider silks by inserting antimicrobial motifs into their sequence to create a chimer that can prevent microbial infection after surgery or implantation [128].

8. Market and industrial production of spider silk

A wide variety of recombinant spider silk proteins are manufactured on a laboratory scale for research. Still, due to their unique properties and applications, the market demand for their industrial production is high. Therefore, many efforts have been made to produce these recombinant biomaterials in different hosts and platforms on a commercial scale. By 2012, the annual global market for technical fibers had already reached approximately $133 billion. In Table 3, some commercialized spider silks are listed.

To produce recombinant spider silk proteins, various eukaryotic (such as tobacco, goat, and silkworm) and prokaryotic hosts have been used [129,130]. The most commonly used prokaryotic host is *E. coli*, which is suitable for large-scale production [62,131].

The sequences used to produce recombinant spider silk fall into two categories: an exact copy of the natural protein [131] or engineered ones according to favorable motifs [132]. Recombinant production of this protein faces challenges due to its high molecular weight and repetitive sequences. The most important of these challenges are genetic deletion, reduction of gene length [133], improper mRNA folding and forming secondary structures [131], poor tRNA pool as well as low solubility of spider silk [134].

To solve these problems, some attempts have been performed, including codon optimization, metabolic engineering of bacterial hosts, meeting the high need of bacteria for glycine and alanine tRNA pools, and fusion of a small ubiquitin for overexpression of heterologous proteins [26,135,136]. To overcome low solubility of the silk, organic solvents like guanidine hydrochloride, HFIP, or FA are used, but these solvents, due to their toxicity, must be entirely deleted in biological applications [131,137]. Oxidation and reduction reactions have also been used to prevent and promote assembly, respectively, in a controlled manner [138].

9. Concluding remarks

Over time, scientists and experts have been discovering more diverse and widely used aspects of spider silks that are cost-effective and safe options for being used in various industries. These applications are far-reaching and range from musical instrument components such as violin strings to surgical sutures and other industrial and biomedical applications. Hence, we have been observing an increase in demand for this useful substance in the industry, prioritizing the production of spider silks on a large-scale. Extracting natural silk from spiders is time-consuming and almost impossible that cannot be responsive to this high demand in industry and biopharmaceutics; moreover, further modifications of silk proteins are required to become safe and ready to apply. To overcome this, biotechnologists have made numerous efforts to produce spider silk proteins recombinantly in highly efficient and rapid growth hosts. Recombinant spider silks can be produced in eukaryotic cells such as plants and animals like goats; however, they pose many challenges like taking up much space for their housing and difficulties in transportation, feeding, watering, and maintenance. On the other hand, in the production of silks in prokaryotic cells, since the high molecular weight and large repetitive DNA sequences of spidroins, several obstacles are associated with this goal in terms of low rate of expression, increased possibility of degradation, so on that might be tackled by some techniques including codon optimization, metabolic engineering of the host, or fusion of a small ubiquitin. The other main concern is whether these recombinant spider silks have properties as excellent and practical as natural. These issues can be somehow resolved by performing different optimizations on these biopolymers. In addition,

Table 3
Some of the top companies and their products using spider silk.

Property	Company name			
	Kraig Biocraft Laboratories	AMSilk	Spiber	Bolt Threads
Expression host	*Bombyx mori*	*Escherichia coli*	*Escherichia coli*	Yeast
Product	Monster Silk™, Dragon Silk™	Biosteel	Spiber's Brewed Protein™ materials	Microsilk, B-Silk™ Protein
Application	Transgenic lines for polymers, Protective textiles	Medical biotechnology products such as breast implants, cosmetics, aerospace, automotive, sports, textiles	Clothing, Sports equipment	Clothing, Beauty treatment
Country	America	Germany	Japan, Thailand, America	America
Established date	2006	2008	2007 (Japan) 2018 (Thailand) 2020 (America)	2009
Production cost (/kg)	$300	$137,500	$37,500	$37,500
Total cash investment	~$5M	$90M	$135M	Not reported

special genetic and chemical modifications are applied to spider silks to obtain more efficient and functional silks on a larger scale. Incredibly, unique characteristics relevant to spidroins have facilitated distinct assembly procedures that enable their recombinant development into various morphologies with particular applications and structures, including fiber, film, non-woven, foam, hydrogel, sphere, and capsule.

The significance of spider silks is not limited to recent centuries and dates back to thousands of years ago; when no chemical drug was available to cover wounds, our ancestors used spider silks for this purpose. Nowadays, scientists have proved the role of spider silk-based wound dressings in skin regeneration while having an antibacterial activity that prevents the infection of injured skin. These coatings are mainly made of non-woven spider silk, and they can accelerate skin regeneration by stimulating the growth of skin keratinocytes and fibroblasts. The applications of spider silks have also drawn the attention of experts in tissue engineering; their flexibility and unique strength properties, while imitating the ECM traits, stimulating cell differentiation, and many other characteristics mentioned in this review comprehensively, have made it an ideal biopolymer for tissue engineering scaffolds. In this regard, researchers have examined the role of spider silk-based scaffolds in engineering tendons, cartilage, bone, and teeth tissues. Notably, they have found that tissues grown on these scaffolds generally develop a morphology almost identical to the natural kind, which is excellent merit brought up by them.

What is more, spider silk advantages have motivated scientists to utilize them for promoting the repair of tendon tissue according to their tensile and improved fatigue properties; in this regard, they are applied as a suitable bone-tendon connector to enable tendon replacement. According to studies, genetically-engineered spider silk chimeras containing functional motifs such as VTK, BSP, DMP1, and R5, can facilitate biomineralization in bone tissue engineering. Applications of spider silks in tissue engineering are not limited to these and can also be traced in the engineering of cardiovascular and neural tissues to treat some severe diseases, since the existence of promising studies that have demonstrated the almost similar traits of spider silk-based engineered cardiovascular and neural tissues to natural ones. It has been discovered that using RGD-spider silk hybrids in cardiac tissue engineering can stimulate the proliferation of cardiomyocytes, and subsequently, these cardiac cells can present an acceptable contraction. In vascular disorders, recombinant spider silk hybrids can be used as scaffolds for the regeneration of blood vessels. Engineering of neural tissue is of high importance down to limited access to autologous transplants and severity of traumatic nerve damages; therefore, creating conduit constructs based on spider silks that can be served as frames that support axonal growth and respond well to external stimuli can make great strides in neural tissue engineering. Not to mention, the biocompatibility of spider silks has made them appropriate biomaterials as bioinks in 3D bioprinters.

It is noteworthy that spider silks are even suitable as carriers in drug and gene delivery due to their superior properties such as hydrophobicity, homogeneity, adjustability, and non-toxicity. On the debit side, it has been observed that not all the drugs/genes can be carried via silk-based carriers, and improper release rate or insufficient loading are among issues that seek more optimizations. Despite all the benefits of recombinant spider silks production, there is a long way to go to achieve the ideal conditions and the challenges at each production stage, such as the affordable way of protein purification and the proper formation of the proteins secondary structure must be overcome. Another critical issue is the low solubility of spider silks and solvents used in order to tackle that (mostly organic solvents like HFIP). The toxicity of organic solvents poses a threat in tissue engineering and biomedicine, and there are no confirmed, efficient and affordable alternatives to be used on a large-scale. Table 4 summarized some applications of the spider silk in biomedical issues.

More investigations are needed to find new approaches for applying spider silks in biomedicine safely. Despite all these, like any other material, spider silks are at the beginning of their development, which requires many studies in this path.

10. Further studies

As described in this review, this advantageous biomaterial is currently widely being used in biomedical applications since they have extraordinary mechanical properties and are made up of proteins that allow scientists to add efficient motifs to them to promote their efficacy. These motifs can enhance its adjuvant property, the proper immune response and, efficient recognition of target immune cells in the field of vaccine delivery. The advantages of this biomaterial are not limited to this and also expand to promoting differentiation of stem cells by addition of particular signaling and adhesion motifs, constructing permanent implants like artificial bone through reduction of silk biodegradability, and producing safe cosmetics, thread lifts, hydrogel form as a lip filler used in beauty surgeries, and burn healing products.

To achieve these advantages out of silk proteins, still, we are facing challenges in modification processes waiting for more research to maintain a balance and safety on properties of spider silk-based materials such as the harsh experiment conditions involved in chemical modifications and change or loss in partial intrinsic characteristics of original silk protein [66]. One of the spider silk applications requiring further investments is developing silk-based biodegradable microfluidic platforms to remove metabolites and detect hazards. Also, the biocompatibility of silks can be utilized to develop organ on-chips in drug delivery studies and toxin testing [139]. Additionally, by altering some production procedures and gene sequence or adding functional motifs to spider silk proteins, or using proper solvents, it is possible to prevent the formation of β-sheets, obtaining a stable solution from that, as what spider silk does and stores it in its glands. This stable solution can make

Table 4
Summarized list of some biomedical application of various types of spider silks.

Spider silk	Evaluated cells/ biopharmaceutics	Application	Results	References
Nephila Edulis native dragline silk	Keratinocytes and fibroblasts	Wound dressing	Antimicrobial properties, cell growth support, Proper biodegradability	[174]
Recombinant spider silk proteins pNSR16 and pNSR32	Rabbits	Wound dressing	Upregulated expression of basic fibroblast growth factors, synthesis of hydroxyproline	[175]
Araneus Diadematus native egg sac	Human articular chondrocytes	Cartilage tissue engineering	Proper porous structure, mimicking natural cartilage Ecm	[176]
Araneus Diadematus spider silk hybridized with tags	Mc3t3 subclone E1 mouse preosteoblasts	Tendon tissue engineering	Lead to mineralization, improve calcium phosphate formation	[177]
Nephila Pilipes dragline silk	_	Humanoid robotic hands	High toughness, conductivity, and good flexibility	[178]
Spider silk with VTK domain	hMSCs	Bone regeneration	Cell growth and proliferation support, increasing crystalline hap formation and osteogenic differentiation	[179]
Recombinant Masp1from *Nephila Clavipes* spider/bone sialoprotein	hMSCs	Bone regeneration	Cell growth and proliferation support, increasing crystalline hap formation and osteogenic differentiation, providing functions as an organic glue, and increasing the stiffness and toughness	[180,181]
Recombinant spider silk protein eADF4(C16)/RGD	Neonatal rat heart cells	Cardiac tissue engineering	Cell growth and proliferation support, form compact cell aggregates, make thick layers, and had faster contractions	[182]
Recombinant spider silk pNSR32/PCL/gelatin	Macrophages	Vascular tissue engineering	Excellent hemocompatibility, facilitated cell growth	[183]
Native spider silk fibers from *Nephila* with vein grafts	Adult rat Schwann cells	Peripheral nerve regeneration	Axonal re-growth and regular alignment, schwann cell migration and proliferation	[184]
Recombinant Masp1	Rat embryonic primary cortical neurons	Neural tissue engineering	Neuronal growth, axon extension and network connectivity support	[185]
Recombinant spider silk protein eADF4(C16)/hydrogels	BALB/3t3 mouse fibroblasts	As Bioink for 3D bioprinting	Non-immunogenicity, cytocompatibility	[186]
Recombinant spider silk protein eADF4(C16) from *A. Diadematus*	Various drug molecules	Drug delivery	Sufficient hydrophobicity, high loading efficiencies, slow biodegradability	[187]
Recombinant spider silk protein eADF4(C16) from *A. Diadematus*	B-galactosidase	Drug delivery	Mechanically stable, protective against proteolysis, allowing external triggers to activate enzyme precursors/intermediates inside the capsules	[188]
Hybrids of silk Ms1 (from *N. Clavipes*)-Her2 binding peptides	Doxorubicin	Drug delivery	No toxicity, proper targeting, biocompatibility and biodegradability	[189]
Ms2 from *N. Clavipes*	Mitoxantrone, etoposide, doxorubicin	Drug delivery	Morphology and colloidal stability, controlled particle size	[190]
Recombinant Masp1/30 Lys/ tumor homing peptides	Plasmid DNA	Drug delivery	No toxicity, proper in vivo targeting	[191]

spider silk conceivable for further uses, such as sprays and eye drop or local injections to repair tissue damages. Another future use of this substance could be to store some sensitive substances such as enzymes, vaccines, and some drugs for a long time and to be used as a container to transfer them safely. All these undeniable and potential advantages of spider silks require extensive studies in the future in attempts to provide suitable and safe spider silk-based biomaterials to be used in drug delivery, industrial products, and regenerative medicine.

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Declaration of competing interest

The authors declare no conflicts of interest.

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