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Review

Spider Silk: an excellent candidate as toughest biomaterial

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Abstract

Spider silk is stupendous tough biomaterial on earth which consists almost entirely of large proteins. It is biocompatible, biodegradable, has high tensile potency, and flexibility. Spiders produce seven different types of silk for specific function. Dragline silk of the golden skin orb-weavers, *Nephila clavipes* and *Araneus diadematus,* are the strongest silk fibres, having high potency and high breaking power. Collecting natural spider silks from webs is a very complicated and prolonged process because of spider's aggressive nature. Therefore, recombinant DNA technology is the most feasible solution to produce spider silk at large scale. Numbers of biomaterials are created using recombinant spider silk such as films, scaffolds, non-woven meshes, hydrogels etc. Spider silk contains molecules that are known to have antimicrobial and hypoallergenic properties. The complete biodegradability of the spider silk makes it more beneficial than that of man-made fibres.

Keywords: dragline silk, tensile potency, flexibility, biomaterials, biodegradability.

1. Introduction

Spider silk is nature's marvelous fibrous biomaterial which contains large proteins. Silks are created by many insects such as mites, parasitic wasps, dragonflies, mayflies, silkworms and spiders. Glycine (Gly), alanine (Ala) and serine (Ser) are principal components in the silk. They can undergo irreversible conversion of soluble protein into insoluble fibres [1-3]. Silks formed by these insects have lots of functions such as providing shelter, protection of eggs or prey wrapping, nest building, web formation, safety lines, protection of offsprings etc. Humans have been using silk as biomaterials more than thousands of years. Silk of silkworm *Bombyx mori* is easy to farm. It has been used commercially from 5000 years for biomedical sutures and in fabric production. It has toughness of 6× 10^4Jkg^{-1} as well as tensile power of about 0.5 gigapascals (GPa). Silkworms have only two silk producing glands. On the other hand, spiders have seven silk producing glands. Each gland produces specific type of silk named as major ampullate silk (also known as dragline silk), minor ampullate silk, flagelliform silk, aggregate silk, pyriform silk, aciniform silk, and cylindriform silk. Spider silk fibre has more tensile strength as compared to steel. It brings out a toughness which is twice to thrice times than that of other fibres like Nylon or Kevlar. Earlier studies have revealed that dragline silk of the golden skin orb-weaver, *Nephila clavipes* and *Araneus diadematus* produced durable silks having high potency and high breaking power [2, 4-7]. Due to cannibalistic and aggressive behavior of spider, they cannot be cultivated as silkworm can be. Collecting natural spider's silks from webs is a very difficult and time-consuming process. To make 1.2 million spiders silk naturally, approximately 8 years time required. Therefore, recombinant spider silk production is the most feasible solution to produce spider silk at large scale. Bacterium *Escherichia coli* is well-established host and commonly used for the recombinant spider silk protein production at industrial scale [6, 8]. Apart from that a variety of heterologous host systems have been used to produce the recombinant spider silks including: yeast (*Pichia pastoris*), plants (soybeans, Arabidopsis), insects (silkworm), transgenic animals (mice, goats), and mammalian cell lines (Hampster), and There are a variety of biotechnological biomaterials constructs from the recombinant spider silk proteins such as production of films, scaffolds for tissue engineering, hydrogels formation, biomedical sutures and artificial nerve constructing fibres. Recombinant spider silk protein is almost similar to natural spider silk protein [6, 8, 9-12]. As compared to bacteria, yield observed for the recombinant silk production in transgenic animals was bare minimum. Presently, large scale production of recombinant silk proteins from transgenic animals is quite costly and challenging in animal breeding. Recombinant spider silk protein production by using *E. coli* is very easiest and quickest method. The use of yeast and mammalian cell lines created elongated, complex protein strands of recombinant spider silk but at less quantity [6, 11, 13, and 14]. In this review, we will discuss about types of spider silks as well as biomedical applications of recombinant spider silks biomaterials.

2. The framework of spider silk

Spider silk is made up of proteins from non-polar, non essential amino acid and hydrophobic amino acids (Gly, Ala and Ser). These fibres are natural polymers which are made up of three domains such as non-repetitive N-terminal domain, repetitive core domain which influence the protein chain and C-terminal domain [6, 7]. The molecular mass of the spider silk proteins vary from 70 to 700 kDa. The dragline silk from *N. clavipes* is characterized by polyalanine and Gly-Gly-X regions, where X is often tyrosine, glutamine or leucine [2, 15-18]. Major Ampullate and flag silks have near about 4 oligopeptide motifs which are repeated a number of times 1. (GA) n/ (A) n, 2.GPGGX/GPGQQ, 3. GGX (X= A, S or Y) and 4. Spacer sequence that holds charged amino acids (Fig.1) Flagelliform silk is usually rich in GPGGX and GGX motifs, and preferably folds into β-turn structures[5,20-22].

A female golden skin orb-weaver spider is capable to create seven different types of silks in seven specialized glands of spider. Major Ampullate silk is used for the frame and radii of a spider's webs. Minor ampullate silk is used in auxiliary spiral and internet reinforcement. The minor ampullate and major ampullate glands have morphological resemblance. The flagelliform silk is used to make capture spiral. Aggregate silk of spider provides sticky coating on capture spiral. Aciniform silk is used to wrap prey and for egg case. The flagelliform, aggregate and aciniform silks are produced from abdominal glands. Pyriform silk is used for attachment of silk and cylindriform silk is also known as egg case silk [6, 7, 12-16, 22-25].

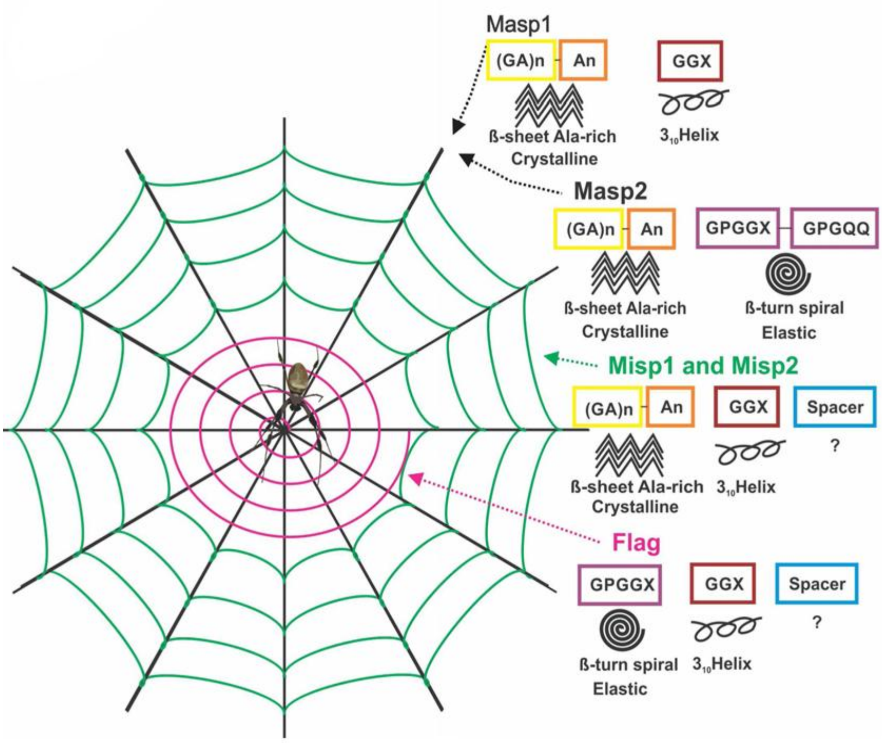

Fig: 1 Diagrammatic representation of the golden skin orb-weaver *N. clavipes* which shows three important spider silk proteins as well as their structures. Colored zones indicate structural motifs in silk proteins and blue colored "spacer" zone marked as "?" because secondary structure of that zone is unclear.

Note: major ampullate spider protein 1 (MaSp1) and major ampullate spider protein 2 (MaSp2); minor ampullate spider protein1 (MiSp1) and minor ampullate spider protein 2 (MiSp2) **[6].**

3. Physical properties of spider silk

The physical properties are the key features attracting researches to spiders silk. Orb-weaver spiders use minimum quantity of silk to grab prey. The web has ability to stop a quickly airborne insect nearly instantly, so that the prey becomes intertwined and captured. Spider silk and the web are constructed for each other. Dragline silk is an exclusive biomaterial. It is stronger than current synthetic fibres. Spider silk is five time stronger than steel Table 1. It has a power of 1.3 GPA, and inflexibility of $16{\times}10^4$ Jkg^{-1} [5, 20, 21, and 26].

Table No.1 Comparisons of physical properties of spider silk with synthetic fibres [27].

S.No.	Fabric	Potency (Nm^{-2})	Flexibility (%)	Power to break (Jkg^{-1})
1.	Major ampullate (dragline silk)	4×10^9	35	1×10^5
2.	Flagelliform silk	1×10^9	>200	1×10^5
3.	Minor ampullate	1×10^9	5	3×10^4
4.	Kevlar	4×10^9	5	3×10^4
5.	Rubber	1×10^6	600	8×10^4
6.	Tendon	1×10^9	5	5×10^3
7.	Nylon	7×10^7	200	6×10^4

Interesting feature of major ampullate silks is their marvelous contraction when exposed to water. Depending on the spider type and other factors, these silks will contract to 50% or less of their original length in water. Spider silk are able to absorb three times more energy than Kevlar, one of the most vigorous materials in terms of weight. Kevlar is para-aramid synthetic fibre. It is well-known to possess a high modulus along with stupendous mechanical potency which is appropriate for use in numerous applications, such as bulletproof vests and tire cords. Spider silks have balanced combination of power and flexibility, and therefore under certain situation mechanically exceed other natural fibres as well as man-made yarns [4, 7, 8, 24, 26-30].

4. Types of spider silk

Spiders are distinctive because of the use of silks throughout their life span. There are seven different types of silks which are produced in specific glands of spider such as major ampullate silk, minor ampullate silk, tubuliform silk, flagelliform silk, aggregate silk, pyriform silk and aciniform silk. Spiders build their webs and carry out a broad range of tasks including: arresting a fall, wrapping a prey, building a web, making egg cases Table 2 [25, 29, 30].

Table 2 Types of Spider silks and their purposes [27].

S.No.	Types of spider silks	Purposes
1.	Major ampullate (dragline)	Net framework and radii
2.	Minor ampullate	Web support
3.	Flagelliform	Core fibres of adhesive spiral
4.	Aggregate	Adhesive silk of spiral
5.	Cylindriform	Egg case silk
6.	Aciniform	Prey wrapping and egg case
7.	Pyriform	Attachment cement and joining fibres

4.1 Major ampullate silk proteins

The golden skin orb weaver spider *N. clavipes* produces nature's toughest silk. It has diameter between 1 to 20 nm. The protein complex is made up of major ampullate dragline silk protein 1 (MaSp1) and major ampullate dragline silk protein 2 (MaSp2). The main dissimilarity between MaSp1 and MaSp2 is the existence of proline residues. MaSp1 is proline free while MaSp2 have 15% amino acid [9, 33, and 34].

4.2 Minor ampullate silk proteins

N. clavipes produced minor ampullate silk in the minor ampullate silk gland of the spider. Minor ampullate silks are primarily composed of two major proteins i.e., minor ampullate silk protein 1 (MiSp1) and minor ampullate silk protein 2 (MiSp2). MiSp1 contain glutamate in low amount and there is absence of proline [35-37].

4.3 Flagelliform silk proteins

N. clavipes produced flag silk in the flagelliform silk gland. It is extremely stretchy and is useful in the production of capture spiral. It contain only one main protein i.e., flagelliform silk protein 1 (Ff1) [33, 35].

4.4 Pyriform silk proteins

Pyriform silk gland produces sophisticated protein superglue (pyriform silk) which is used to fix the major ampullate scaffold to a trees or walls (Fig. 2). It contains two types of proteins, which are named as spider coating peptide 1 (SCP-1) and spider coating peptide 2 (SCP-2). SCP-1 and SCP-2 contain 36 and 19 amino acids [33, 35].

4.5 Aggregate silk proteins

It has also shown that the aggregate glands produce two different proteins, aggregated glandular silk factor 1 (AgSF1) and aggregated glandular silk factor 2 (AgSF2). Aggregate glands have been postulated to manufacture spider glue protein glue proteins for silk fibres that join to form sticky droplets, which interact with the capture spiral silk and influence the mechanical properties of the spiral filaments [15, 23, 33, 34, 35, and 37].

4.6 Aciniform silk proteins

Aciniform silks are used by spiders for prey wrapping, construct sperm webs and for web decoration. It has just one protein, aciniform silk protein 1 (AcSp1). Silk produced by aciniform silk gland are used in number of purposes such as, to reinforce the pyriform silk cement matrix as well as for providing soft coating to the egg case [33, 35].

4.7 Tubuliform (cylindriform) silk proteins

Tubuliform silk is formed by female orb-weaver spiders during reproduction time to build egg cases. It contains only one protein, tubuliform spider protein 1 (TuSp1). It has moderately high tensile potency. TuSp1 is secreted by tubuliform silk gland [35, 38, and 39].

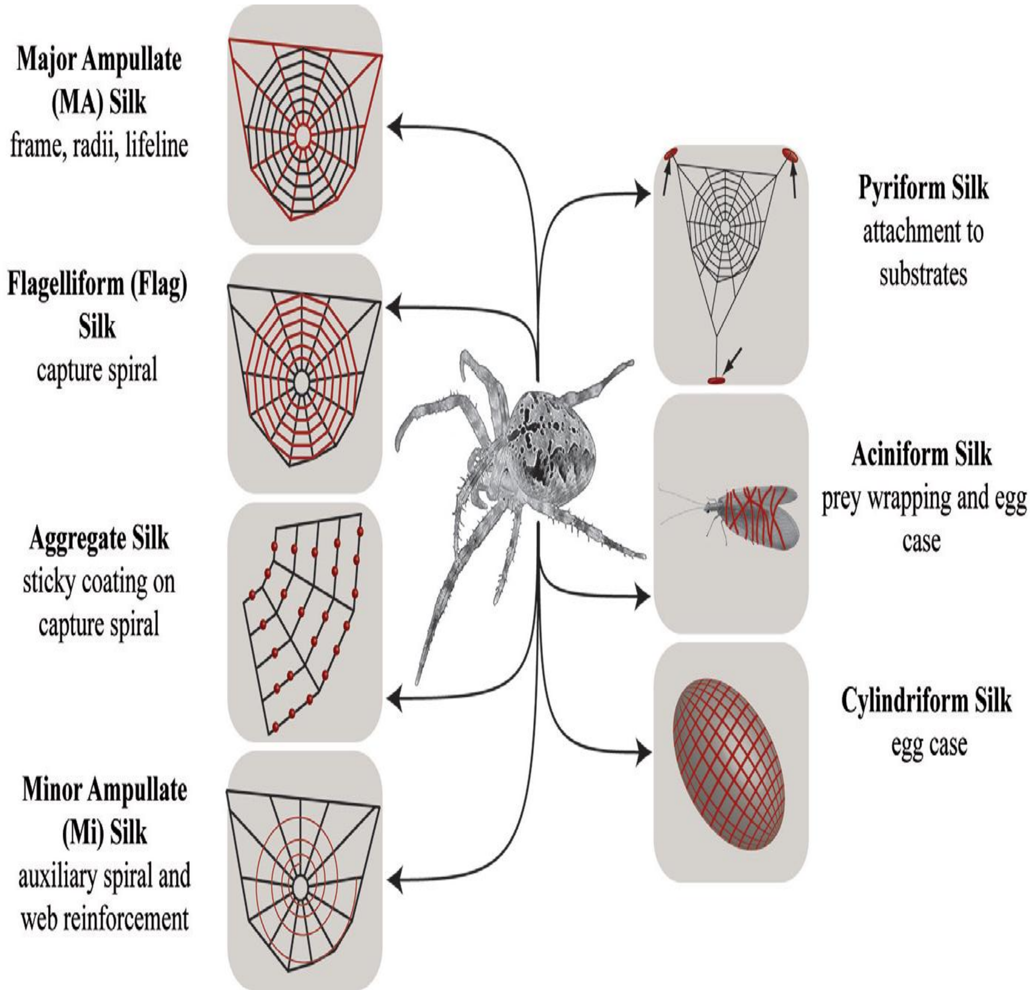

Fig: 2 Illustration of different types of spider silks and their specific purpose in spider [7].

5. Production of recombinant spider silk proteins by using different host systems

Biotechnological construction of recombinant spider silk is the absolutely feasible method to produce silk at large scale in addition to fulfill the growing desires of medication and biotechnology. As a result, production of spider silk was followed through recombinant DNA technology. This method consists of five main steps: (1) Digestion of cloning vector (2) insertion of linker to modify cloning vector (3) Ligation of spider silk monomer into vector (4) transformation into host (5) expression and purification of recombinant spider silk [6,40].

5.1 Single-celled organism used as host

Single-celled organisms (bacteria and yeast) are used as host for the production of recombinant spider silk. *E. coli* is gram-negative; rod shaped bacterium and is inexpensive. It is well-established host for scale up of spider protein at industrial level. It is very simple to handle and has short doubling time. For the production of recombinant spider silk proteins in host *E. coli*, a commercially available vector pET30a (+) was used. This vector was modified by inserting linker segment into it (Fig.3). Linker segment contains restriction sites, Nhe1 and Spe1 while vector consists of Xho1 and Nco1 restriction sites. Linker was inserted into the Xho1 and Nco1 sites of pET30a (+) and is converted into pET30L. In the next step, spider silk monomer is then inserted into vector then transformed into bacterial host for expression. For the purification of the protein, N-terminal labeling is used with metal affinity chromatography [6, 40, and 41].

Yeast *(P. pastoris)* is used as host to produced *N. Clavipes* spider dragline silks. It produced long repetitive proteins. Mammalian cell lines, such as baby Hampster kidney cells, bovine mammary epithelial alveolar used to express MaSp1 and MaSp2 [6, 42]. Use of *E. coli* for formation of recombinant spider protein is most feasible method. But use of yeast and mammalian cell lines produced longer, complex proteins but in less amount [6, 11, 15].

5.2 Multicellular organism as host

Silkworm *(B. mori)* is used to produce recombinant spider silk protein. According to researchers, in the silkworm, total 6 mg of spider protein was expressed. Infection of *Baculovirus* leads to production of large quantities of proteins in a short period of time (Tokareva *et al.,* 2013; Wen *et al.,* 2010). Transgenic lines of tobacco and potato were engineered to express major ampullate spider proteins MaSp1 genes from *N. clavipes.* It was found that the yield of recombinant silk proteins in transgenic animals is lower as compared to bacteria Table 3. Nowadays, productivity of recombinant silk proteins from transgenic animals is difficult task and expensive too [6, 44].

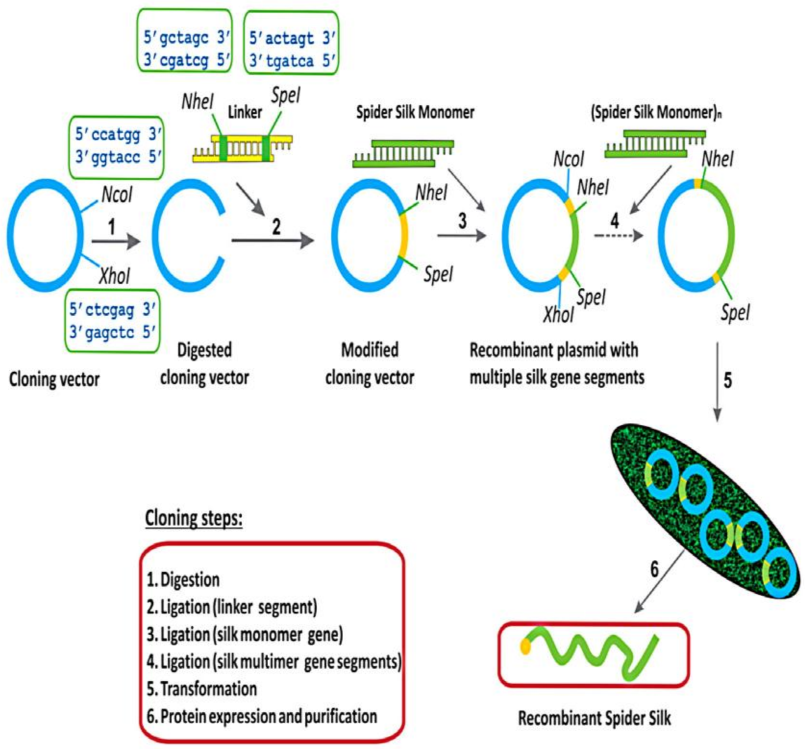

Fig: 3 Production of recombinant spider silk proteins using pET30a (+) vector [6].

Table 3. Productivity of recombinant spider silk proteins in multiple hosts [6, 8].

S.No.	Host category	Host	Origin	Cloning plasmid	Yield
1	Yeast	*Pichia Pastoris*	*Nephila clavipes*	pBR322 derived *PstI*	663 mg/L spider silk protein produced, which is 16% of total proteins
2	Plant	Tobacco and Potato *(Nicotiana tobaccum, Solaum tubersum)*	*Nephila clavipes*	pUC19	Spider silk protein produced up to 0.5% of total proteins
3	Animals	Transgenic mice	*Nephila clavipes*	pGEM-5zf	11.7 mg/L to 25–50 mg/L of spider silk protein Produced
		COS-1 cells	*Euprosthenops sp.*	pER1-14	
		Baby hamster kidney	*Araneus diadematus*	pBSSK+	
		Baby hamster kidney	*Nephila clavipes*	pSecTag-C	
4	Insect	*Bombyx mori*	*Nephila clavipes*	pSLfa1180fa	Spider silk protein produced up to 40% of total proteins
5	Bacteria	*Salmonella typhimurium*	*Araneus diadematus*	pET30L	0.7–14 mg/L of ultra-high molecular weight spider silk protein produced with the productivities of 1–12 mg/L/h
		Escherichia coli	*Nephila clavipes*	pET30a(+)	500–2700 mg/L spider silk protein Produced

6. Biomedical applications of recombinant spider silk biomaterials

Spider silk is amazing natural construct, which has incredible mechanical properties. It is biocompatible, eco-friendly, has high tensile power and great flexibility. Natural spider silk is very complicated to obtain in large quantities due to aggressive behavior of spider. Biotechnological applications of recombinant spider silk biomaterials include coatings, films, hydrogels, non-woven meshes, drug carriers and medical sutures (Fig.4). They offer the highest potential for spider silk, due to the admirable mechanical properties and biocompatibility. Silks assemble in various morphological shapes as well as films and hydrogels, reflective excellent 2nd or 3D scaffolds for tissue engineering and tissue repair [4, 6, 7, 8, and 33].

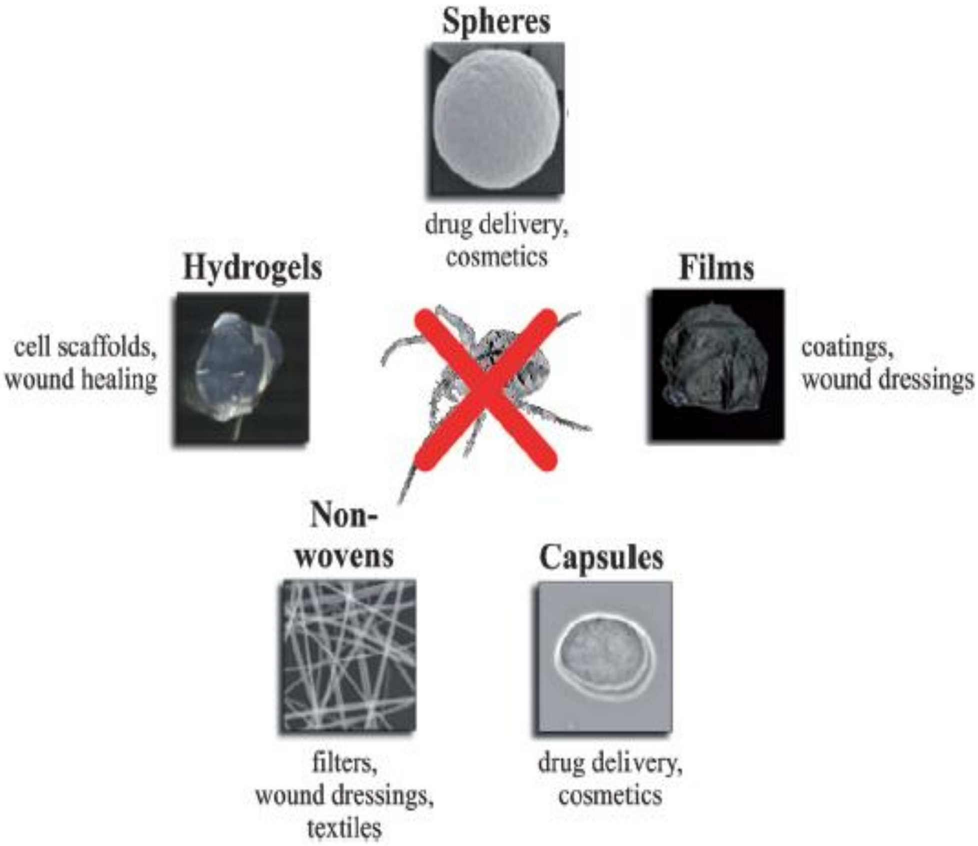

Fig 4: Recombinant spider silk proteins biomaterials along with their prospective applications [7].

Coatings are created with recombinant spider silk proteins that enhance biocompatibility in materials such as medical grade silicone implants which can reduce inflammation and rejection. Spider silk can also encapsulate drugs, proteins, genes and cells for delivery or diagnostics [6, 11, and 23]. Recombinant spider silk particles can be used as gene carrier for tumor cell-specific delivery as well as used in implantation and inoculation (Fig.5). Scaffolds were used to test cell repair [15, 16].

Silk fibres have been used as sutures for injuries from several years because of their wonderful strength, biocompatibility and least immunogenicity. Non-woven mats are very interesting biomaterial because they can increase surface area and provide rougher topography for cell attachment. Endothelial cells when cultured on top of silk fibres, they adhere to the surface and proliferate better [45-46].

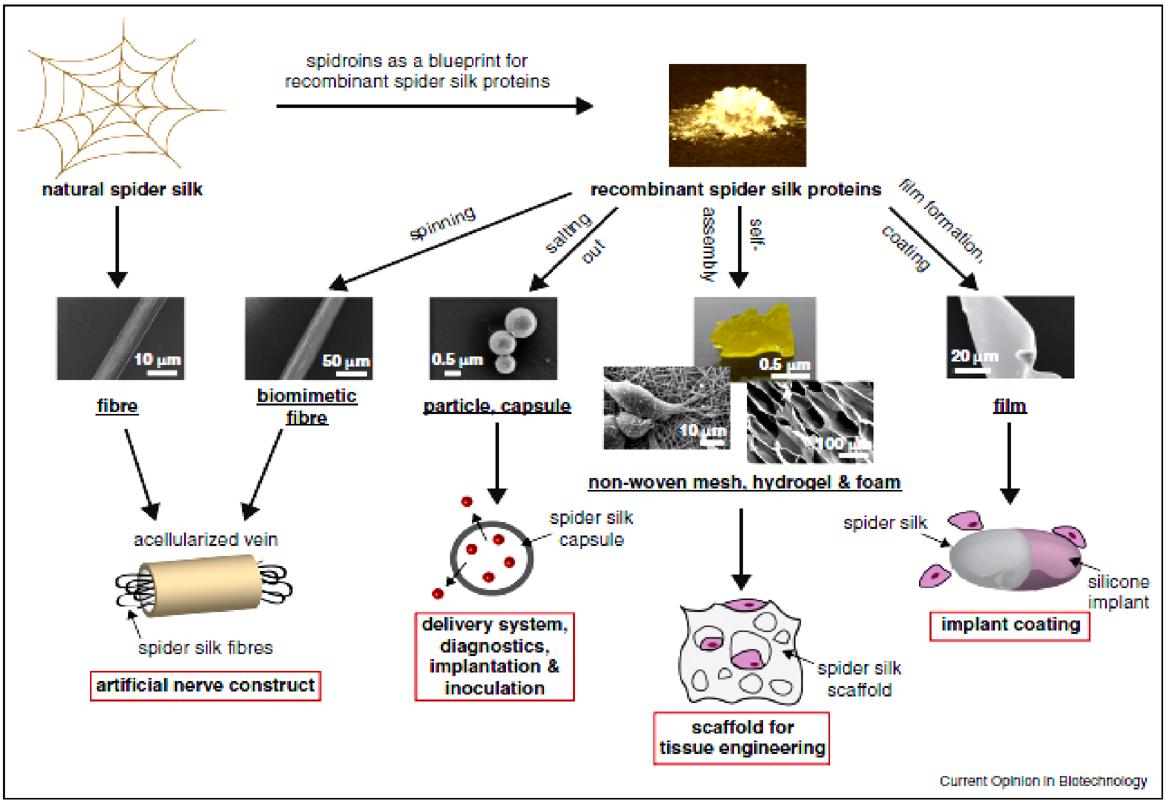

Fig: 5 Applications of recombinant spider silk in biomedical sciences [23].

7. Conclusion

Spider silk is incredible biomaterial having flexibility and high strength. Biomaterials created from it have range of applications such as tissue repair, synthetic nerve construction, drug carrier. Spider Silk has the prospective impact on the materials science, engineering and as well as on the green chemistry.

8. Future perspectives

Spider silks are made up of biopolymers that have evolved from hundreds of years. Recombinant spider silk proteins have various technical and biomedical applications. There are lots of possible future uses of recombinant spider silk biomaterials such as bulletproof vest, tough clothing, parachutes and construction of buildings. Other potential applications of silks in the field of cosmetics for the production of shampoos, soaps, cream and nail varnish, etc.

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