Silk Fibroin and Spidroin Bioengineering Constructions for Regenerative Medicine and Tissue Engineering (Review)

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The review is about the developments of modern bioengineering constructs from two unique biopolymers: the main protein of silkworm silk, fibroin, and frame silk of spider web, spidroin, and their applications in regenerative medicine and tissue engineering. Both types of polymers possess such important properties as biocompatibility, biodegradability, high strength and elasticity. Availability of silkworm cocoons in nature, debugged methods of fibroin purification make this protein very perspective in bioengineering constructs. A spider web protein, spidroin, is less common in nature, but the development of alternative methods for its production makes it a promising biopolymer.

The structure and properties of silk fibroin and spidroin, their advantages over other natural and synthetic polymers, technologies of biopolymer construct fabrication are considered in this review. Silk fibroin and spidroin are shown to be applied for creation of 3D matrices promoting regeneration of damaged organs and tissues, biodegradable cell carriers and pharmaceutical preparations.

Key words: silk fibroin; spidroin; bioengineering constructs; regenerative medicine.

The selection of material for regenerative medicine as well as the technology used for making biostructures of particular materials depend on the nature of the application: for use with bone tissue, blood vessels, skin, muscular tissue, nerve fibers etc. To be used successfully the biomaterial must have certain chemical, biological and mechanical properties [1, 2]. Among these required chemical properties one can include the lack of harmful chemical reactions with tissues and interstitial fluids, plus resorption at a controlled rate inside the body [3]. The necessary mechanical properties are the durability of the structure and the possibility of performing surgery with it. The principle technological characteristic of the material is its biocompatibility with the organism. The main protein of silkworm silk (fibroin) and the frame silk of spider webs (spidroin) both share these advantages.

Structure and properties of the biopolymers

Silk fibroin. Fibroin is the main silk protein obtained from the cocoons of silkworms and related species. It is a heterodimer consisting of two chains, covalently bound through disulfide bridges [4, 5]. The glycated protein P25 with a mass of 30 kDa is united with Fib-L and Fib-H by hydrophobic bonds [6]. It is thought that this complex is formed of 6 heavy and 6 light chains per molecule of glycated P25 protein [7]. The light and heavy chains of fibroin, as well as protein P25, are coded separately in the genome [4]. The primary sequence of fibroin consists of glycine (43%), alanine (30%), and serine (12%) [8]. There are smaller amounts of tyrosine (5%), valine (2%), aspartate, glutamate, and cysteine, which perform the main role of integrating the different subunits into a single molecule. Glycine, alanine and serine comprise the main structural sequence Gly-Ala-Gly-Ala-Gly-Ser of the heavy chain Fib-H — 70% of all the protein sequence [9]. Similar sequences also occur: Gly-Ala-Gly-Ala-Gly-Tyr (20%), Gly-Ala-Gly-Tyr-Gly-Ala (6%), and Gly-Ala-Gly-Ala-Gly-Ala (4%), forming the basis of 12 regular hydrophobic crystalline blocks, the length of each being 413 amino-acid residues. These 12 blocks constitute 94% of the total protein sequence and they are separated by 11 intermediate, irregular, amorphous sections with lengths of 42–43 negatively charged amino-acid residues [8].

Natural silk does not dissolve in water or in dilute solution of many acids or alkalis, but it is soluble in concentrated solutions of lithium chloride, lithium thiocyanate, and calcium chloride. As fibroin can form α-spirals and β-folds, it exists in several structural forms: the 1st is loose and globular, it is unstable and mechanically lacking durability; the 2nd is rich in α-helices and amorphous forms (silk I) and is strong and elastic; the 3rd is the crystalline β-form (silk II), providing the highest tensile strength and resistance to strong mechanical shocks, although it is less elastic than the α-form [8]. Fibroin can preserve its crystalline structure for long periods [10]. The form of the protein saturated with β-structures determines and maintains the structure of implants created from silk fibroin, providing both integrity and stability in aqueous solutions of the type found in the physiological medium of the body [11].

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For this reason, structures including fibroin should be exposed to β-crystallization in cultivation medium or under *in vivo* conditions prior to use. Fibroin comprises 70–80% of the protein mass in each silk thread; the rest being sericin, which acts as a glue fastening the fibroin fibrils together to form the cocoon, together with low percentages of fat-like and wax-like substances, plus inorganic anions and cations (less than 1%) [12, 13].

Fibroin is a thermostable protein; its denaturation temperature being higher than 127°C. The elastic modulus of fibroin is 15–17 GPa, and the protein has a high tensile strength (610–690 MPa). Fibroin is highly transparent, its transmissibility in the visible spectrum is 90–95%, while the refractive index of fibroin films is 1.55 at a thickness of 30–50 µm [14].

Fibroin is used in regenerative medicine as a material for making matrices [15, 16] and films [17], and is included in pharmaceuticals used for the delivery of medical and biologically active substances into the body [18, 19]. Furthermore, it has an antimicrobial effect, so it can be recommended as a novel, but natural antibacterial biomaterial [20].

Spidroin. This is the frame silk of spiders' webs [21]. The best-studied properties are those of webs made by spiders of the *Nephila family*, represented by *Nephila clavipes*, which spin webs of various types [21, 22]. From the main ampullary glands the spider produces the web's frame silk the threads of which are very elastic and characterized by high tensile strength, comparable with that of Kevlar and stronger than steel of equivalent dimensions, making this material unique among natural, and the majority of artificial, materials [23]. Spider web silk is resistant to environmental conditions, has high biocompatibility but is biodegradable. These properties result from the structure of the material. The frame thread includes two covalently bound proteins: spidroin 1, which is coded by the gene *MASP1* (forms crystal-like β-folded structures), and spidroin 2, coded by the gene *MASP2* (forms an amorphous matrix).

Spidroins are large proteins: the molecular mass of isolated spidroins is 300–350 kDa, and, in their dimeric forms — 550–650 kDa [24]. Spidroins have a periodic structure with a large number of direct repetitions [25]. The primary sequence enriched by polyalanine and glycine-rich sequences, forms a heterogeneous secondary structure with segments of crystal-like structure consisting of antiparallel β-folded regions interspersed with less structured parts of the so-called amorphous matrix [26]. This matrix, rich in glycine, is represented by spiral parts and occasional loops, but is not unstructured, as its macromolecules are directed mainly parallel to the vertical axis of the spidroin fibrils [27].

There are two types of crystal-like domains: densely packed and strictly oriented β-folded and more freely located folds. 90% of the alanine residues take part in the formation of the crystalline domains, and the β-folded structures are located parallel to the fiber axis. In highly ordered areas, the methyl groups of the alanine residues are oriented strictly at an angle of 90° to the chain axis. In less-ordered areas, the methyl groups of the alanine residues do not have a strict orientation to the fiber axis and have a higher possibility of spatial re-orientation [27]. Crystalline parts of the macromolecule are responsible for its high tensile strength while the amorphous matrix is responsible for its elasticity. The crystalline domains and amorphous matrix do not have clear structural borders. A spidroin macromolecule is capable of structural transitions decreasing or increasing its crystallinity. Changing the conditions of silk-spinning can affect these transitions [28]. The frame threads of a spider web have high thermostability (up to 230°C). At 210°C, the melting point of the main crystal-like domains, their limit of tensile strength is equal to 22 GPa, the elastic strength is 1.1 GPa and the relative lengthening is 9% [29]. Due to the presence of defects and fluctuations in the thickness of the threads, the mechanical strength of these natural proteins can vary. The structure of a spider web thread can change with time: a year after its production it has better mechanical strength and elasticity, but further exposure to aging results in the disintegration of amine groups, and, therefore, after longer storage, its strength and elasticity decrease [30]. Ultraviolet radiation also leads to aging [31]. Environmental humidity affects the degree of hydration of the threads and influences the protein's mechanical properties by changing the character of the hydrogen bonds between the protein chains [32]. At the maximum degree of hydration, 2/3 of the thread mass is water which is bound within the main amorphous matrix [22]. Humidity has a positive effect on the tensile parameters and elastic modulus.

There are recombinant analogs of natural spidroin which have been obtained by synthesis in yeast cells, *Pichia pastoris* and *Saccharomyces cerevisiae*, mammalian cells and with the use of transgenic animals and plants [33–35]. These recombinant analogs differ from the natural materials in their lower mechanical strength, although this can be increased using a range of chemical and mechanical methods — by stretching the threads in methanol [36] or by crystallization [37]. The introduction of additional polynucleotide sequences in the genes coding for spidroins can also help modify the properties of the recombinant proteins [38, 39]. Co-polymers differing from the native proteins can be created, based on the recombinant analogues. Due to their properties spider web frame proteins are used for making products for use in regenerative medicine [40].

For a complete understanding of the uniqueness of these two biopolymers and an objective evaluation of their advantages it is necessary to review other polymers and compare them with fibroin and spidroin.

Synthetic polymers

Biodegradable synthetic biomaterials based on polymers of organic substances were among the first to be used in the field of tissue engineering.

Polyglycolic acid (PGA) is a biodegradable thermoplastic polymer, the simplest linear aliphatic polyester [41]. One of the advantages of this material is the ability to link additional chemical chains, leading to the appearance of new properties that increase its range of application. Due to its crystalline structure, PGA is insoluble in water, and its plasticity increases with increased humidity, allowing for easy formation of biostructures [42]. At present PGA is widely used for the creation of surgical suture material [43, 44]. Surgical sutures from PGA do not need to be extracted after surgery. They completely dissolve in the body within a few months, to form carbon dioxide and water.

Polylactide is an aliphatic polyester, the monomer of which is lactic acid. It displays thermoplasticity, is biodegradable and biocompatible [45]. Biodegradable packaging, personal care products, screws, plates and pins for fixing fractures, and surgical threads can be made of polylactides [46]. They can is also used for producing medications as antimicrobial substances encapsulated with polylactide at appropriate concentrations are gradually released, preventing the growth of microorganisms [47]. Additionally, 3D printing can use polylactide as a source material [48].

Polycaprolactone is a biodegradable polyester, a polymer of ε-caprolactone. Polycaprolactone is resistant to water, solvents and various oils, as well as being characterized by low viscosity. It is also easy to process. When it is mixed with starch this reduces its cost while improving the biodegradability [21]. In medicine polycaprolactone is used for suture materials [49], and as a carrier for the delivery of medications into the body [50], while, in cosmetology it is used to make fillers [51, 52].

Polyethylene terephthalate (PETP) is bioinert thermoplastic, and in an amorphous state it is a solid, colorless and transparent substance, while in its crystalline state it is opaque and white [53]. When it is heated to its glass transition temperature it becomes transparent, and the material remains in this state after quenching. The length of the PETP polymer molecule affects its viscosity, and the higher the viscosity, the lower its rate of crystallization [45, 54]. PETP is almost insoluble in water and organic solvents, resistant to acids and weak solutions of alkali, is fairly strong and durable and is a dielectric. By changing the chemical composition of the side-groups of the polymer molecules one can produce materials with different degradation rates [54]. In medicine PETP is used for making prostheses of bone vessels [55], tendons, ligaments and heart valves [56].

Polyamides are plastics based on linear, synthetic high molecular mass compounds. In their melted state aliphatic polyamides have a low viscosity across a small temperature interval [57], but have high melting points. Polyamides are characterized by their hydrophilicity [58], and their water absorption affects their impact resilience and strength [59]. Surgical sutures and prostheses of blood vessels are often made from polyamide fibers [60, 61].

Polyurethanes are heterochain polymers which are synthetic elastomers and contain unsubstituted or substituted urethane groups [62]. Polyurethanes are resistant to acids, mineral oils and oxidants; they are hydrolytically more stable than polyamides. In medicine polyurethanes are used for making implants [63, 64], catheters and tubes for general use [65], surgical drapes and wipes.

Silicones are high molecular mass, oxygen-containing organosilicon compounds including polyorganosiloxanes (silicon oils, water repellents, and low molecular mass rubbers) and organo-silicon monomers (silanes) [66, 67]. Silicones can be divided into three groups on the basis of their molecular masses, degree of crosslinking and the type and number of organic groups in the silicone molecules: silicone liquids — having less than 3,000 siloxane links; silicone elastomers — containing from 3,000 to 10,000 links; and silicon resins — having more than 10,000 siloxane links together with a fairly high degree of crosslinking [68, 69]. Silicones have a wide range of properties. They can increase or decrease the adhesion of proteins and cells, can make materials hydrophobic, preserve their properties under extremes and rapid changes of temperatures, they have dielectric properties, are chemically and biologically inert, elastic, ecologically-friendly and non-toxic [70, 71]. Products made from silicones are resistant to nuclear radiation, ultraviolet radiation, and electric fields.

Natural polymers

When bioconstructions are to be made, polymers of natural origin have considerable advantages over synthetic ones. The decomposition products of these materials in the body are natural metabolites taking part in the biochemical processes inside cells; therefore the biocompatibility of natural polymers is much higher. Their mechanical properties are similar to the properties of products made of synthetic polymers.

Collagen is a fibrillar structural protein of the intercellular matrix and is particularly widespread in the bodies of mammals (25–35% of the total amount of protein in the body) it is the basis of connective tissue and makes this elastic and durable. A collagen molecule is a right-handed spiral of three α-chains, each turn of which includes three amino-acid residues. Due to the numbers of arginine-glycine-aspartate sequences (RGD-sequences) in the primary structure, collagen is a biomaterial able to provide for cell adhesion [72, 73]. It has low allergenic properties and is non-toxic, but its unregulated and rapid biodegradability considerably restricts the period of functioning of collagen products to less than 1 month. When injected into the body collagen stimulates repair processes [74], contributing to the generation of the body's own collagen, but it does not provide complete regeneration of the organ because it soon dissolves leaving scar tissue. The formation of a heterogeneous supramolecular gel structure containing collagen [75, 76] enables this biodegradation to be slowed. Collagen has hemostatic properties, so it is used for the preparation of artificial valves and vessels [77, 78].

Gelatin is the product of collagen hydrolysis. Porous gelatin tubes [79] are used as substrates for mature mesenchymal stem cells and for rat hepatocytes [80]. It has been established that the implantation of microspheres made from gelatin enables growth of cerebral neurons, skeleton myoblasts and cardiomyocytes to take place [81].

Chitin (poly-N-acetyl-D-glucosamine) is nitrogen-containing polysaccharide composed of N-acetyl-glucosamine residues, joined by beta-[1,4]-glycoside bonds. Chitin is the most widespread natural polysaccharide. It is the main component of the exoskeletons of many invertebrates [82], but is also found in the cell walls of fungi and bacteria, where it performs protective and supporting functions [83]. Chitin is a rigid semi-transparent polymer, insoluble in water, resistant to the effects of dilute acids, and alkalis, and to alcohol and other organic solvents.

Chitosan is an aminopolysaccharide of 2-amino-2-deoxy-β-D-glucan formed by the deacetylation of chitin. Chitosan can bind many organic water soluble substances, for example, bacterial toxins, because of its ability to form hydrogen bonds. In dissolved form it is a highly effective sorbent [84]. Fats, fat-soluble compounds, and saturated hydrocarbons can be bound by chitosan due to its molecular sieve effect and its hydrophobic interactions. Microbial enzymes such as chitinase and chitobiase can split chitin and chitosan to N-acetyl-D-glucosamine and D-glucosamine respectively, so they are ecologically friendly materials. Chitin and chitosan are used to create artificial blood vessels, catheters, suture materials, wound- and burn-healing dressings and adhesives [85] as well as being formed into structures for the delivery of medications [86, 87]. Chitosan can be made into strong and durable products in the form of films, sponges, and membranes [88], however, they are thermally unstable, which makes their sterilization more difficult. The inclusion of additional components to form composite matrices decreases their lability — the use of plasticizers such as glycerol, lauric acid, lactic acid and polyethylene glycol [89].

Bacterial polyhydroxyalkanoates — polyhydroxybutyrates (polymers of β-hydroxybutyric acid) are in a class of intracellular polyesters from the heterocysts of cyanobacteria [90]. Polyhydroxyalkanoates come in a variety of chemical compositions and structures and therefore differ in their physical and chemical properties. The best-studied are the homogeneous, polyoxybutyrate, and the bicomponent copolymers of oxybutyrate and oxyvalerate, or oxybutyrate and hydroxyoctanoate. Their physio-chemical properties are similar to polyethylene and polypropylene, but they are biocompatible and biodegradable. The decomposition products of these polyesters (carbon dioxide and water) are non-toxic. They are resistant to ultraviolet radiation, have high gas barrier properties, good water resistance, and thermal stability. Depending on the composition of the copolymers used with the polyhydroxyalkanoates, their mechanical properties change [91]. In the field of tissue engineering, polyoxybutyrates are used, for example, in the preparation of porous bioresorbing scaffolds [92].

Alginic acid is a polysaccharide which is obtained from red seaweeds (*Laminaria japonica Aresch*). Alginic acid is a viscous substance, insoluble in many organic solvents; one of its components can adsorb 300 parts by weight of water, so it is an effective thickener [93]. Sodium alginate, potassium alginate, and calcium alginate are used as food additives. In dentistry alginates are used for taking elastic impressions. Inorganic fillers (zinc oxide, talc) comprise the main mass of ‘alginate powder’ and affect the viscosity of the material and its strength after solidification [87, 94]. Alginates are used in systems for the delivery of biologically active substances [95], in addition to providing means for the injection of cells and various factors through the formation of hydrogels able to interact with divalent and trivalent cations (Mg^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Al^{3+}, Fe^{3+}) [96, 97]. Alginates do not contain RGD-sequences, therefore the adhesion of cells on their surfaces is rather low. Nevertheless, porous sponges made from alginates enhance the restoration of nervous conductivity of rats [96], and alginate matrices improve the regeneration of cartilage tissue [98].

None of the polymers is universally applied. For example, polylactides, polyamides, and polyurethanes are good for the production of prostheses and medical equipment; chitosan and collagen are more suitable as additives for the manufacture of tissue-engineering structures; while polycaprolactone and polyglycolic acid are used for the manufacture of high-quality sutures.

By contrast, the value of silk fibroin and spidroin is that they have properties that make them practically universal materials for use in tissue engineering, pharmacy and medicine, regardless of the type of structure required (matrices [99], films [100], sutures, microspheres or as microcarriers of medications). Unlike the majority of polymers of synthetic and natural origin, products made of silk fibroin and spidroin have high nanoporosity which is important for the biological properties of the finished products [101]. They are, simultaneously, both biocompatible and biodegradable, strong, but relatively easy to use, while not generally requiring the addition of many supplementary materials to produce the product needed. Unlike polyhydroxybutyrate and polyglycolic acid which decompose to carbon dioxide and water, the products of fibroin and spidroin decomposition are amino-acids, thereby supplying additional building materials for tissue regeneration. Both these biopolymers contribute to the adhesion and proliferation of cells on their surfaces which does not occur with many other polymers (for example, alginate). The modification of silk fibroin and spidroin with additives can increase the effectiveness of their application [102].

Technologies used in the production of biopolymer structures

Several different technologies are used for making three-dimension matrices, each having advantages and disadvantages. The method is selected depending on the properties of the materials used, and the desired characteristics of the final structure and the field of application.

Leaching method. This is based on the leaching principle, where one of the system components, namely a blowing agent, is washed out, leaving behind a porous three-dimensional matrix. Blowing agents can be liquid droplets and powdered materials: wax, salts (for example, sodium chloride, ammonium carbonate) [103], sugar [8]. The size of the blowing agent particles affects the final size of the structure's pores. The main advantages of the method are its simplicity, universality and its convenience in controlling the sizes and forms of the pores. A disadvantage is the limitation on the thickness of the final construction (up to 3 mm) and the difficulty in the formation of a matrix with guaranteed pore interconnectivity [104].

Sublimation method. In this method, the polymer is dissolved in a solvent at an appropriate concentration, then the solution is frozen and the solvent is extracted by lyophilization under vacuum. Thus, a highly porous matrix having internal pore interconnections can be formed [105]. The pore size can be controlled by the rate of freezing and the pH; a faster rate of freezing allowing pores of smaller sizes to be obtained [106]. However, one of the main advantages of the method is that it does not require either high temperatures, or a separate stage of leaching. Its disadvantages include the length of the process and the limitation of obtaining only pores of small size. This technique is applicable to different polymers including silk fibroin, polyglycolic acid and polylactide [107].

Electrospinning method. Electrospinning involves forcing the polymer solution through a small-diameter needle into space with a high voltage electrostatic field, resulting in the formation of filaments of up to 1 µm diameter on a metal collector. The main advantage of this method is the ability to make matrices with high surface to volume ratios, to orient the polymer threads and to regulate the porosity of the matrices and the thickness of the fibers. More than two hundred different polymers including silk fibroin [108], collagen [109], chitosan [110] and gelatin [111] are suitable for use with this method.

Bioprinting method. This uses technology based on inkjet printing to form 3D structures with pre-set morphology. The "Bio-ink" can consist of live cells and proteins while the "biopaper" is the polymer substrate, providing and stabilizing the framework of the structures being formed [112]. At the end of the process an incubator is used in which the matrix is fixed under specific conditions, and the cells can be grown and proliferated if the bioprinting of cell-containing structures has been performed. The method of bioprinting does not have many of the drawbacks of traditional methods of matrix formation, as it can immediately form the final structure required. The accuracy of the method and its high reproducibility allow for layered printing and the application of the growth factors and cytokines required for cell adhesion and differentiation on the resulting construction [113]. Thus, bioprinting is an up-to-date, contactless, non-destructive method that can be used for printing two-dimensional and three-dimensional structures layer-by-layer [114, 115].

Use of silk fibroin and spidroin in tissue engineering and regenerative medicine

Strong and elastic films, three-dimension matrices and tubes can be made of regenerated *Bombix mori* silk fibroin. *In vitro* studies have shown that such constructions maintain the adhesion and proliferation of eukaryotic cells, and their structure is beneficial for the even distribution of the proliferating cells, both on the surface and deep within the matrix. *In vivo* experiments with the subcutaneous implantation of such matrices into mice have shown the high degree of biodegradability of constructions of silk fibroin and their ability to undergo neovascularization [116]. Matrices made from recombinant spidroin have also shown their compatibility with cell culture, providing for adhesion and proliferation over long periods of time, while also having low immunogenic activity [117].

Regeneration of bone tissue. To eliminate defects of bone tissue, Varkey et al. [118] made three types of matrices using silk fibroin: nanofibrous matrices made using electrospinning and sponges and porous films both made by lyophilization. These were studied as substrates for the adhesion and proliferation of osteocarcinoma MG-63 cells. The results showed that all three types of bioconstructions were biocompatible and contributed to cell attachment.

Sangkert et al. [119] created matrices modified with collagen and fragments of decellularized tissue formed by freezing-thawing. The experiments showed that these matrices are potentially useful for bone tissue engineering and the treatment of the cleft palate ("wolf jaw").

Matrices of silk fibroin with hydroxyapatite have been used for the adhesion and proliferation of MG-63 osteocarcinoma cells. Shao et al. [120] showed that these nanostructured constructions have excellent biomimetic and mechanical properties, maintain the adhesion and proliferation of cells, while functionally contributing to biomineralization. The fibroin biomimetic matrices with added hydroxyapatite contain three layers: one is of cartilage with longitudinally oriented microtubules; another is a bone layer with a 3D porous structure, and the intermediate layer has a dense structure that can effectively maintain the regeneration of cartilage and bone tissue *in vivo* [121]. *In vitro* experiments with composite matrices from silk fibroin (40%) and chitosan (60%) with more than 90% internal three-dimensional porosity showed that such structures sustain fast adhesion, growth, and proliferation of MG-63 cells, have good biocompatibility and only biodegrade slowly, while helping the cells to secrete cytokines to build the extracellular matrix [122]. When gelatin and hydroxyapatite are added to *in vitro* matrices of silk fibroin the adhesion of murine embryonic fibroblasts and their proliferation in 3D culture are increased, indicating that such multicomponent constructions could be promising in the field of regenerative medicine, especially when bone tissue is to be restored [123]. Matrices of silk fibroin and chitosan with the addition of vascular endothelial growth factor contribute to the proliferation and activity of embryonic human osteoblasts [124]. Fibrous hydrogels of silk fibroin and sodium alginate enable crystals of hydroxyapatite to be obtained, having the required morphology for restoring bone tissue [125].

Membranes of biologically active glass and silk fibroin can maintain cell proliferation and affect the odontoblastic differentiation of dental stem cells from human tooth pulp. These membranes can be used as tissue-engineering film material for the regeneration of the pulp-dentine complex [126].

Matrices of strontium-doped calcium polyphosphate, with the addition of dopamine and silk fibroin, implanted *in vivo* effectively accelerate the process of mineralization and the regeneration of new bone tissue in rabbits. Immunohistochemical investigation has also shown that such matrices increase the secretion of vascular endothelium growth factor (VEGF) and the main fibroblast growth factor (bFGF) [127].

Porous matrices of silk fibroin form suitable niches required for the long-term survival and functioning of implanted stem cells of rat bone marrow for the regeneration of bone tissue *in vitro* and *in vivo* [128].

Mineralized silk fibroin resembles natural bone in structure, the cellular and mineral layers of fibroin being crucial for regeneration of the bone tissue. The investigators noted that the ability to contribute to spondyloarthrosis was increased when mineralized silk fibroin was cultivated with bone marrow stromal cells [129].

Restoration of cartilaginous tissue. Matrices of silk fibroin containing a mechanical growth factor, transforming growth factor and stem cells can contribute to the regeneration of articular cartilage *in situ* [130].

In the study by Vishwanath et al. [131] it was shown that the most suitable matrices for tissue restoration are those made of silk fibroin with the addition of chitosan in a 4:1 ratio. Other matrices with corresponding ratios can contribute to adhesion, the survival rate and proliferation of cell cultures (for example mesenchymal stem cells obtained from placental blood) while the detection of glycosaminoglycan secretion onto these matrices testifies to their ability to accelerate the regeneration of cartilage tissue. Composite matrices from collagen and silk fibroin in a 7:3 ratio with the addition of polylactide-co-glycolide microspheres contribute to the restoration of articular cartilage and the integration of the regenerating tissue into the cartilage surrounding it. The effectiveness of these structures has been shown *in vivo* and *in vitro* by experiments with matrices implanted into an artificially made defect of the articular cartilage in rabbits [132].

Wound healing. *In vitro* and *in vivo* experiments with rats showed that matrices of silk fibroin with the addition of gelatin microspheres encapsulating the antibiotic gentamycin had an antimicrobial effect, suppressing *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa*. These matrices enable the gradual release of their active content, providing good wound healing and are therefore effective for treating deep, infected burns and severe burns [133]. Experiments *in vivo* on rats showed that nanomatrices of silk fibroin facilitate the healing of burns and make epithelization faster, as was demonstrated by histological investigations of tissue samples [134]. Patches made from silk fibroin generated using electrospinning and vitalized with mesenchymal stromal cells taken from human adipose tissue, have been used for skin regeneration in mice with diabetes. *In vivo* investigations indicated that non-vitalized patches are as effective for treating diabetic wounds as the patches containing mesenchymal cells. Both types of patch equally stimulated angiogenesis and the synthesis of collagen. At the same time it was noted that the lack of cells on such fibroin patches has considerable advantages, as it reduces the risk of transferring mutant cells or triggering an immune response. Furthermore, non-vitalized patches can be prepared in advance and stored for substantial periods. This is an important step towards more successful treatment of ulcers caused by diabetes mellitus [135].

Human mesenchymal stem cells cultivated on hydrogels of silk fibroin with varying degrees of rigidity and growth factor content to help them differentiate into mature smooth muscle cells, have demonstrated the effectiveness of using silk fibroin for the preparation of tissue-specific matrices for cells [136].

Highly porous films of silk fibroin formed by electrospinning can provide for oxygen delivery to a wound, so are used as bandaging materials. As the average diameter of the nanofibers affects the mechanical and biological properties of these products, they can be manufactured with appropriate features as required [137].

Reconstruction of ligaments. Matrices of regenerated silk fibers with a hierarchical structure including nanofibrils, microfibers and fiber bundles, have mechanical characteristics similar to those of the anterior cruciate ligament. Biodegradation tests showed that such matrices lose 8% of their weight after being placed into sodium phosphate buffer for 60 days and 62% of their weight when in a solution of actinomycete proteases for 48 hours. The authors [138] concluded that their hierarchical structure, mechanical properties, high biocompatibility and biodegradation resistance meant that matrices of regenerated silk are appropriate for use in the tissue engineering of ligaments.

When artificial ligaments are covered with PETP containing silk fibroin their hydrophobicity decreases while their biocompatibility increases. *In vitro* experiments showed that the adhesion and proliferation of mural fibroblasts on the surfaces of these structures are improved compared to ligaments which were not modified with the polymer [139].

Matrices made of collagen sponges and fibroin knitted net can imitate ligament components. *In vivo* experiments on rabbits showed that these matrices are suitable for reconstruction of the anterior cruciate ligament in animals, so they have a potential for clinical use [140].

Biodegradable hybrid nano- and micromatrices consisting of twisted strands of silk fibroin covered with nanofibers of poly-3-hydroxybutyrate or polycaprolactone, contribute to the adhesion and proliferation of mural fibroblasts *in vitro*. The mechanical properties of the hybrid structures can be optimized for the regeneration of various natural ligaments and tendons by changing the number of twisted strands of fibroin [141].

Regeneration of blood vessels. Experiments [142] have shown that fibroin matrices with added heparin inhibit the proliferation of human smooth muscle cells and improve hemocompatibility by releasing heparin for 7 days and contribute to the formation of new vessels after subcutaneous implantation into rats. These matrices can potentially be used as vascular implants, because they have a high degree of porosity (92%), good compatibility with blood and are simple to produce.

Tubular matrices from silk fibroin formed by electrospinning can be used for the regeneration of small-diameter blood vessels. Experiments *in vitro* and *in vivo* on rats demonstrated that these matrices have suitable morphological and mechanical properties, and are biodegradable and biocompatible [143].

The inclusion of porous silk matrices containing channels 254 μm in diameter in the structure, together with vitalization with endothelial cells from the human umbilical vein (HUVECs) promotes quick vascularization and integration *in vivo.* Hollow channels in the matrices enhance the proliferation of endothelial cells and the formation of capillary-like tubes during preliminary incubation *in vitro* [144].

Multifunctional biomaterials made from silk fibroin with heparin allow the controlled release of vascular endothelium the growth factor *in vitro*. The released factor is functionally active and promotes the growth of human endothelial cells. The addition of low-molecular-weight heparin to silk increases its hemocompatibility [145].

Two-layer vascular matrices of small diameter, made from recombinant spider silk, polycaprolactone, gelatin, and chitosan, are biocompatible and biodegradable. They have suitable hydrophilicity and hemocompatibility, and promote the adhesion and proliferation of mesenchymal stem cells *in vitro* and during subcutaneous implantation *in vivo* [146].

Artificial prostheses of vessels made from silk fibroin were studied *in vitro* and *in vivo* in rats for evaluation of their acute and subacute hemocompatibility and compared with commercially available transplants manufactured from PETP. The results of the experiments confirmed the possibility of using silk fibroin for vascular regeneration [147].

Films and matrices of silk fibroin with the addition of N,N'-methylenebisacrylamide are insoluble in water and acids, and have a porosity ensuring appropriate conditions for cell cultivation. These structures are more compatible with blood in comparison with films and matrices made from pure fibroin. By preventing blood coagulation and platelet adhesion they are perfect candidates for use in vascular surgery [148].

Restoration of gastrointestinal and urinary tracts. Two-layer matrices of silk fibroin can maintain the adhesion and proliferation of gastrointestinal tract epithelial cells and human smooth muscle cells as shown experiments *in vitro*. Moreover, these matrices promote the differentiation of primary epithelial cells from the human esophagus in the direction of the suprabasal and surface phenotypes. There are plans to carry out experiments *in vivo* with the application of these matrices to restore organs of the gastrointestinal tract [149]. After the addition of vascular endothelium growth factor, matrices of polyesterurethane covered with silk fibroin and having microchannels in the structure were effective in promoting the regeneration of esophagus muscles, forming a normal histological structure [150].

Matrices from silk fibroin were tested on experimental animal models as a means of restoration of the bladder and urethra. The results showed high biocompatibility, biodegradability and good regeneration of the smooth muscles and urothelium. These matrices have all the necessary biomechanical properties: durability and elasticity [151].

Meshes made from spider web silk obtained from *Nephila edulis*, maintain the adhesion and the growth of primary urothelial human cells without substantially changing their properties. This means that the material is suitable for testing in preclinical investigations for bladder reconstruction [152].

Films made from silk fibroin and keratin show improved mechanical properties if gelatin is added to them. After being mixed with sodium peroxide the films have been shown to maintain a high level of oxygen around them for two weeks and to promote enhanced cell growth. This biomaterial has antibacterial properties. Experiments on animals showed the appropriateness of its application for the reconstruction of urinary tract defects [153].

Regeneration of nerve tissues. Fibers from regenerated silk fibroin with graphene oxide are biocompatible and have mechanical properties which allow their use for the production scaffolds for the restoration of bones, and for the growth and regeneration of nerve tissue. These matrices can act as electrodes for storing energy while forming a biocompatible substrate for "electronic skin" [154].

A tissue-engineered nerve channel matrix based on silk fibroin and collagen was created to co-cultivate seed cell material using Schwann cells and adipose stem cells. *In vivo* experiments on rats indicated that these structures improve the regenerative microenvironment and accelerate the regeneration of peripheral nerves [155].

Bioengineering tissue from porous silk fibroin sponge preliminarily seeded with rat brain neurons in a soft collagen matrix can imitate native nervous tissue [156].

Bioengineering nerve channels made from silk fibroin and polylactide-co-glycolide obtained by electrospinning, have high porosity, hydrophilicity, tensile strength and biocompatibility. *In vitro* and *in vivo* subcutaneous implantation in rabbits restores peripheral nerves, making these channels appropriate for use in clinical conditions [157].

Fibrous membranes of polylactide-co-glycolide and silk fibroin are hydrophilic. The degree of stretching of the strands in these structures can be regulated by changing the percentage of the protein. Laboratory tests showed that the addition of fibroin to polylactide-co-glycolide, enhances the proliferation of nerve cells. Membranes formed into nerve channels and implanted into a defect of the sciatic nerve of a mouse promoted more organized and mature nerve regeneration [157].

Biodegradable carriers of cell cultures and pharmaceuticals

Cell microcarriers. Microcarriers from recombinant spidroin with complex topography of their surfaces provide for effective cultivation of immortalized primary fibroblasts. *In vivo* experiments showed that subcutaneous injections of a microgel suspension in the area of a skin wound do not lead to the development of acute inflammation, but do accelerate the regeneration of tissues and stimulate neurogenesis and angiogenesis in mice [158]. To increase adhesion, microcarriers from an aqueous fibroin solution can be linked with gelatin, forming a hydrophilic biopolymer with an integrin-recognizing RGD-sequence. The resulting bioresorbing microcarriers maintain the adhesion and proliferation of 3T3 murine fibroblasts [159].

Composite membranes made from silk fibroin with the addition of acetamide have good compatibility with cells and the requisite mechanical properties, together with stable long-term optical transparency while contributing to the proliferation of corneal stromal cells *in vitro* [160].

Under *in vitro* conditions fibrous meshes made from recombinant spidroin are suitable for the adhesion and growth of cardiomyocytes without requiring extra covering with adhesive factors (for instance, fibronectin) [161].

Carriers of pharmaceuticals. Sponges made from silk fibroin with gelatin are potential carriers of curcumin and docosahexaenoic acid, releasing them into the body to provide an antitumor effect [162]. Due to their controllable sizes and permeability, biocompatible and biodegradable microcapsules made from silk fibroin with the addition of polycaprolactone are potentially suitable for use as 'intelligent' systems for pharmaceutical delivery [163].

Microspheres made from silk fibroin are also potential objects for the delivery and release of medicaments in the body [164]. Their morphology, size, and polydispersity are regulated by changing the molecular mass and concentration of the silk fibroin, the ionic strength and the pH of the buffer solution. Multifunctional microspheres made from silk fibroin with iron oxide are effective as carriers of doxorubicin hydrochloride (a traditional anticancer medication) [165].

Monolayer and multilayer films made from spidroin are used in pharmacy and in medicine as matrices for the delivery of both low and high molecular weight pharmaceuticals, especially in cases when the mechanical strength of the eluting matrix is of great importance [166]. Biodegradable rods of silk fibroin are promising biocompatible structures for the storage and delivery of anastrozole for treating breast cancer. *In vitro* and *in vivo* experiments have shown them to contribute to the steady slow release of the pharmaceutical, the rate depending on the size of the structures [167]. Nanoparticles from silk fibroin, encapsulating the antibacterial pharmaceutical gentamicin, after being applied on a titanium surface to achieve continuous release of the pharmaceuticals *in vitro*, increased the adhesion of osteoblasts, their proliferation and differentiation compared to the titanium surface with no such covering. This technology provides an effective approach to treatment in the field of orthopedics and dentistry [168]. Porous matrices from silk fibroin and polyvinyl alcohol can be used as wound dressings due to their low cytotoxicity and the appropriate release of pharmaceutical curcumin that can be loaded in them [169].

Microparticles of silk fibroin formed with dispersion-drying or dispersion–lyophilization are suitable for the targeted delivery of the anticancer medication Cisplatin by its inhalation into the lungs. Crosslinking of the fibroin with genipin modifies the release of the active substance to make it more effective. The ability of the particles to form aerosols allows adequate dispersion and delivery of the active substance into the lower respiratory tract [170].

Spheres made from bioengineered spider silk are used as a means of targeted anticancer therapy [171]. They can provide pH-dependent release of doxorubicin, and do not show any cytotoxic effect before the pharmaceutical is loaded.

In vivo, when a hydrogel made from silk fibroin and polyethylene glycol and loaded dexamethasone was injected directly into the membrane of the fenestra cochleae of guinea pigs, the medication showed itself to be an effective and safe means for glucocorticoid delivery and prolonged release in the inner ear [172].

A solution of silk fibroin with riboflavin (as a photoinitiator for covalent crosslinking) forms a transparent elastic hydrogel which can be used for changing the shape of the cornea to restore visual acuity [173].

Nanospheres consisting of silk fibroin and nanodiamonds can be used as carriers of drugs (for example, doxorubicin). The release of these substances is controlled by measuring the fluorescence of the nanodiamonds inside the spheres. These nanospheres are therefore useful nanocomposite platforms for diagnostic and therapeutic purposes [174].

Conclusion

Silk fibroin and spidroin are used in the fields of bioengineering and regenerative medicine as materials for making three-dimension matrices promoting the restoration of damaged organs and tissues and for creating biodegradable carriers of cells and pharmaceuticals. They have many unique features meaning that structures made from these two biopolymers are still being actively developed and studied.

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