Review Article

Understanding Silk, the queen of all fabrics: An excellent biopolymer in biomedicine

Debjani Roy[1]*

[1]Bose Institute, Unified Academic Campus, Kolkata, India

Abstract

Tissue engineering (TE) is a multidisciplinary field involving engineering, biochemistry and biomedicine. TE combines cells with scaffold materials and necessary growth factors to regenerate or replace damaged or degenerated tissue or organ. Since the last few decades the scientists are in the search of natural biopolymers made by living organisms that can be used as a scaffold for tissue formation. Amongst the variety of biomaterials tested, silk fibroin (SF) based scaffold is a promising biomaterial that is being used for bone, cartilage, ligament, tendon, skin regeration. Silks are highly expressed protein polymer produced by silkworms during their pupal stage to protect the worm from the external environmental stress. The FDA approved silk fibroin (SF) is retaining its attention in biomedicine due to ease of its processing, excellent biocompatibility, mechanical properties and non-toxic degradability. Here we present application of the SF-based studies to understand the self-assembly of this protein and the development of advanced drug delivery devices based on SF hydrogel containing silk nanoparticles by controlling the self-assembling processes.

Keywords: Tissue engineering, Biopolymers, Silk fibroin, Drug delivery devices

Introduction

Silk, commonly known as the 'queen of all fabrics', was first discovered in China (*Columbia Encyclopedia*, 2000). Since the last few decades the scientists are in the search of the natural biopolymer in tissue engineering. Biopolymers are a specific class of polymers made by living organisms. Silkworm silk has been commercialized in the textile industry for more than 4000 years, due to its physical properties, i.e., lustre, lightweight, flexibility, and strong mechanical strength. Moreover, silk has been approved by the Food and Drug Administration (FDA) for use in sutures and has been applied to biomedical applications for the last 2 decades. Silk fibroin (SF), extracted from silkworm silk, is a unique natural protein that has been used as a potential biopolymer for TE, due to many desired physiochemical properties such as excellent biocompatibility, biodegradability, bioresorbability, low immunogenicity, and mechanical properties. SF also can be combined synergistically with other polymers to form SF-based composite scaffolds, that can further promote cellular behaviour (e.g., differentiation, proliferation, and attachment). Further to this it is possible to fabricate SF-based biomaterials into various material formats, such as films, hydrogels, sponges, 3D structures, and nanoparticles. Here we present several opportunities of the SF-based studies to understand self-assembly of this protein and its prevention, promising 3D composite scaffold for printing of various human tissues and the development of advanced drug delivery devices based on SF hydrogel containing silk nanoparticles by controlling the self-assembling processes [1].

Origin of Silk and Silk Fibroin

Silks are proteins that are produced within glands after biosynthesis in epithelial cells. In nature over 200,000 different silk-producing arthropods exist. Out of these, there are many silk-producing families such as silkworms, spiders, lacewing, glowworm, and mites, some of which can spin silk into fibers during their metamorphosis (cocoon generation). Silks originating from silkworms and spiders are usually used for biological applications. However, in the case of spider silk once it is spun and contacts air and hardens, which restricts mass production of spider silks. Compared to spiders, the yield of fibers obtained from one silkworm cocoon is around 10-fold that of the gland of a spider. Bombycidae and Saturniidae play the most important roles in silkworm silk research, which feed

Mail id for correspondence
roydebjani13@gmail.com
Received 12 October 2022
Revised 13 October 2022
Accepted 14 October 2022
PHARMAWAVE 2022; 15:44-48.

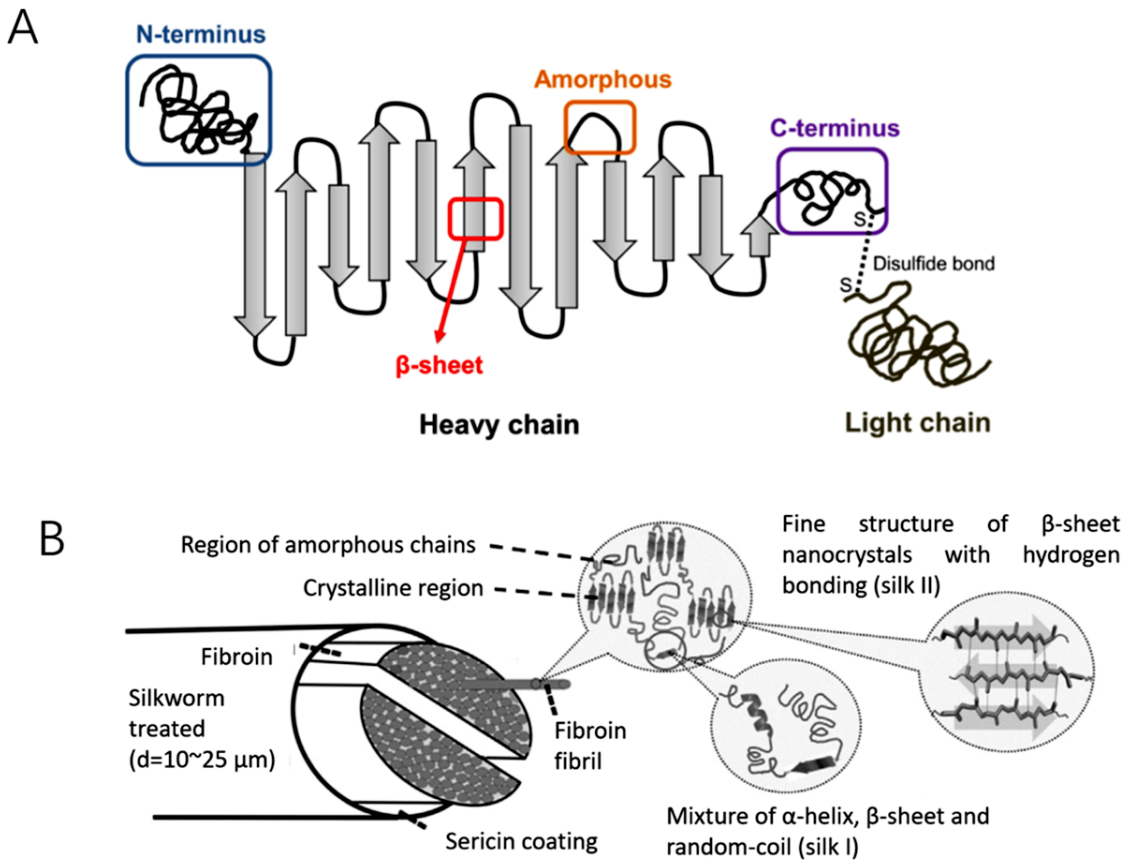

Figure 1. Schematic diagram of the silk structure. 1(A) heavy chain (i.e., N-terminus, β-sheets, Amorphous and C-terminus) and light chain which linked via disulphide bonds. 1(B) silkworm thread, fibril overall structure and silk fibroin polypeptide chains (Reproduced from [1] under the purview of Creative Commons License).

on either the mulberry tree (Bombycidae) or other food sources, the latter being regarded as non-mulberry (Saturniidae) silks. The most common silk originates from *Bombyx mori* (*B. mori*), a mulberry feeding silkworm produces high quality fibers. Over the last 5000 years, *B. mori* was domesticated from an ancestral species in China and has been extensively reared worldwide to obtain its silk. Sericin is removed from the SF fibers by a degumming process, boiling in alkaline conditions. Researchers continuously work on improving the degumming process after degumming, the average diameter of silks fibers was reduced to 10 to 25 μm [1].

Structure of Silk Fibroin

The silk fiber (SF) of silkworm cocoons are natural composite biopolymer comprises of the core protein fibroin (which contributes 70-80% of silk cocoons of silkworm) and the Sericin a second silk protein that constitutes 20-30% silk cocoons of silkworm. (Kundu et al 2008). SF contains two main chains, a heavy (H-) chain (390 kDa) and a light (L-) chain (26 KDa), that are linked via disulphide bonds to form a -L complex (Figure 1). P25 (25 KDa) is a Asn-linked glycoprotein oligosaccharide chain that is hydrophobically linked to H-L complex. The H-chain, L-chain, and P25 are the three polypeptides that are the major components of the cocoon of *B. mori* and exist at a molar ratio of 6:6:1, respectively. The amino acid sequence of the H-chain consists of Glycine (45.9%), Alanine (30.3%), Serine (5.3%), Valine (1.8%), as well as 4.5% of 15 other amino acid types. The Gly-X (GX) dipeptide motif repeats constitute 60–75% of the H-chain. The hydrophobic residues of the dipeptide repeats usually form stable antiparallel β-sheet crystallites. The two hexapeptides occupy 70% of the GX dipeptide motif region, for which the peptide sequences are known to be Gly-Ala-Gly-Ala-Gly-Ser and Gly-Ala-Gly-Ala-Gly-Tyr [1]. silk I is a metastable crystalline structure that includes bound water molecules. Silk II is the most stable state due to strong hydrogen bonding between adjacent peptide blocks, resulting in increased mechanical properties like rigidity and tensile strength. Figure 1 shows the schematic diagram of the silk structure. The secondary structure obtained from regenerated silk fibroin (RSF) solutions contains crystalline and amorphous structures. In a crystalline structure, silk includes β-turns (silk I) and insoluble structures formed by folded β-sheets (silk II), while in an amorphous state silk consists of α-helices, turns and random coil structures. Methanol or potassium chloride can easily convert silk I to silk II. Silk III is the unstable crystal structure of SF, which exists at the air–water interface of RSF solutions. The strong friction between the twisted bundles of nanofibrils is the main reason for the strong interactions, and causes the

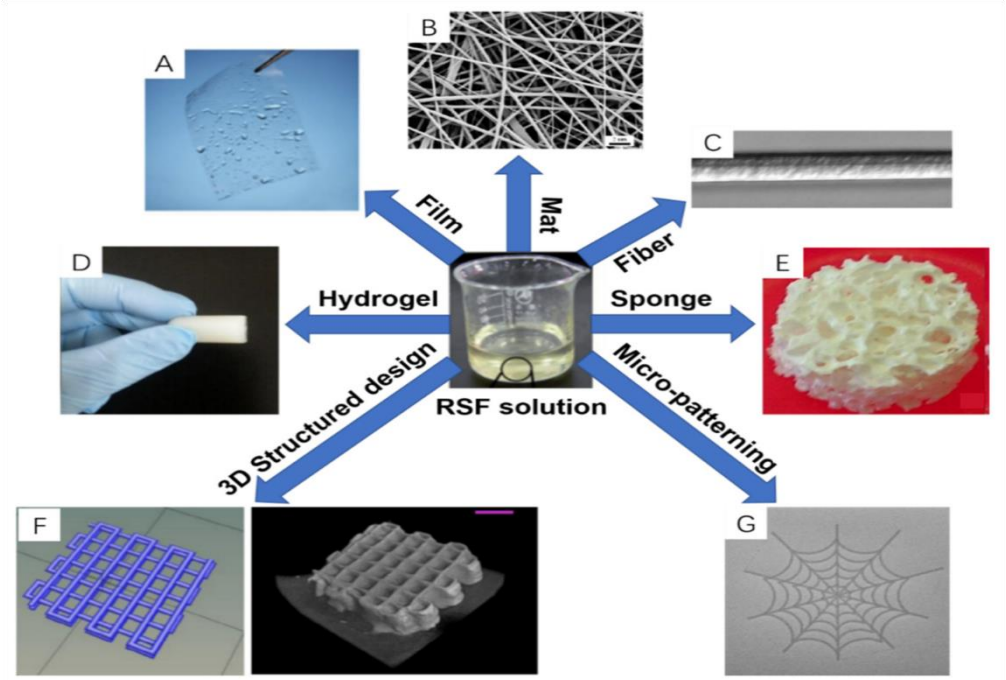

Figure 2. SF-based scaffolds with different representative structures: (A) Film; (B) Mat; (C) artificial fiber; (D) Hydrogel; (E) Sponge; (F) 3D structure design and printed scaffold; (G) Inkjet-printed silk pattern. (Reproduced from [1] under the purview of Creative Commons License).

excellent mechanical strength of silk fibers. SF is a semi-crystalline structured protein whereas sericin is an amorphous protein-polymer functioning as a gumming agent. It has been found that sericin-free fibroin fibers show better mechanical properties than sericin encased fibroin. Additionally, sericin-free fibroin fibers show better biocompatibility in-vitro and in-vivo. Therefore, sericin proteins are often removed from SF to obtain reconstituted silk fibroin (RSF) ensuring biocompatibility in tissue engineering applications [1].

Silk fibroin scaffold: RSF hydrogels

Hydrogels are water-swollen 3D polymer networks, which can be cross-linked via either covalent bonds or hydrophobic interactions as well as electrostatic interactions and hydrogen bonding (Figure 2). These hydrogels are excellent for the implementation of cell seeding and encapsulation in the development of tissue engineering applications. It is possible to encapsulate cells in hydrogels giving them mechanical support in a 3D environment similar to their native tissue. Employing hydrogels as a cell-laden bioinks for the fabrication of 3D tissue constructs enables a lack of immunogenicity, since the hydrogel-based bioink is patient-specific and derived from biopolymers that demonstrate excellent biocompatibility and biodegradability, decreased organ rejection, increased organ viability, and enhanced the supply in accordance to the demand.

To date, RSF hydrogels have been used with increasing popularity alongside other RSF morphologies, which is mirrored by the ever-increasing silk-based publication records [1]. RSF hydrogel formed from reconstituted silk fibroin by a sol-gel transition in the presence of acid, ions or other additives for drug delivery in vitro and in vivo for cell culture.

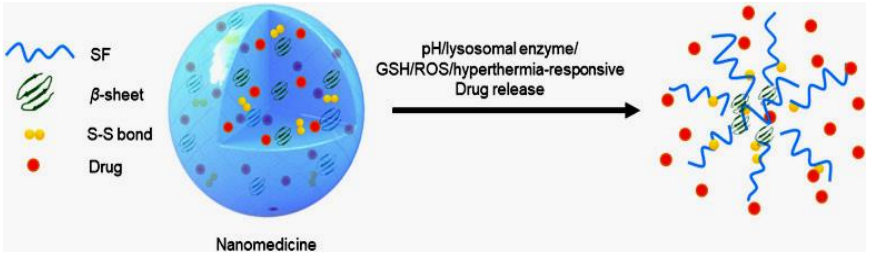

Figure 3. Schematic illustration of multi-responsive properties of SFNPs. (Reproduced from [2] under the purview of Creative Commons License).

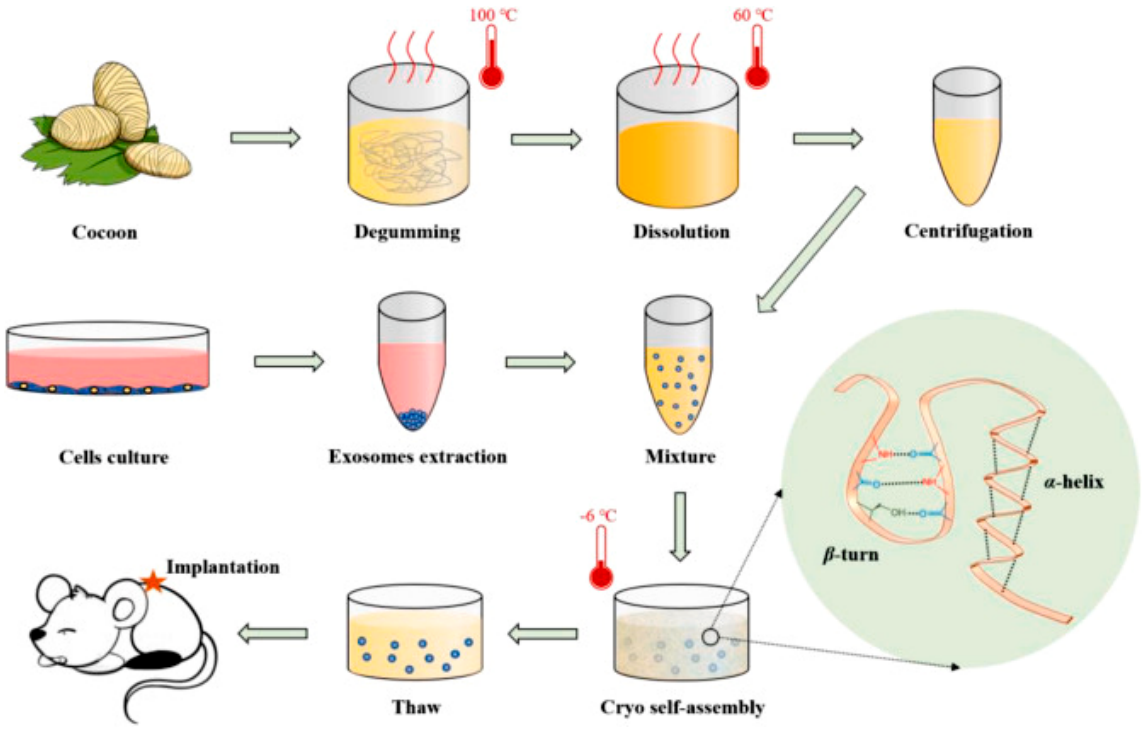

Figure 4. Scheme of the overall study of Cryo-self-assembled silk fibroin sponge. (Reproduced from [3] under the purview of Creative Commons License).

In general, during sol-gel transition of RSF solutions, the SF structural conformation changes from a random coil structure (Silk I) to a β-sheet conformation (Silk II). However, it is important to note that electro-gelation hold an exception to this, where the random coil conformation changes to α-helixes rather than β-sheet and the transition process is reversible by reversing the polarity of applied potential. Cells can be encapsulated into RSF hydrogels that can be consequently used as a delivery system. For example, Wang et al encapsulated human mesenchymal stem cells (HMSC) into sonication-induced RSF hydrogels, and reported proliferation and viability in static cultures after a week of in vitro cultivation [1].

Silk Fibroin-Based Nanoparticles for Drug Delivery: Controlled release device composed of SF micro particles embedded in SF hydrogel:

In recent years, the natural FDA-approved polymer, Silk fibroin has become an attractive natural polymer for drug delivery due to its versatile characteristics such as good biocompatibility, desired biodegradability, large scale production, and self-assembling property (Figure 3). It has been reported that silk-fibroin derived particles due to sustained release augments intracellular uptake and retention of the entrapped drug with the down modulation of more than one pathway. Importantly, they can efficiently load small-molecule drugs, proteins, and nucleic acids through surface adsorption, physical encapsulation, and chemical coupling, which are able to prevent drug degradation, optimize the drug pharmacokinetics, and increase the cellular uptake amounts of drugs. The self-assembly can be modulated by external environmental factors. Under certain external stimulation such as metal ion, low temperature, organic solvent, and ultrasound, the soluble, and irregular Silk I can be transformed into non-soluble Silk II. On the other hand, under high concentration of neutral salt and other certain conditions (e.g., acid, ROS, enzyme, and hyperthermia), the β-sheet structures of Silk II undergo a conformational reversion to amorphous structures of Silk I. Therefore, the transformation between the crystal structures of silk fibroin is a multi-factorial regulated process, which is fundamental to the multi-responsive property of SFNPs. This multi-responsiveness can cause spatial and temporal release of drugs in desired tissues [2].

Kaplan group was the first to report that SFNPs showed an obvious pH-dependent drug release property. The release rate of doxorubicin (DOX) from SFNPs was significantly increased in the buffer (pH 4.5) in comparison with that in the buffers with the pH values of 7.4 and 6.0. They speculated that the loss of the negative net charges in the buffer (pH 4.5) weakened the electrostatic interaction between silk fibroin molecules and DOX, resulting in the accelerated release of DOX from NPs [2]. These results collectively reveal that SFNPs have obvious pH/ROS/GSH/hyperthermia/lysosomal enzyme-responsive properties, which can facilitate the specific drug release in the targeted cells via micro environmental stimuli. Hyperthermia could gradually destroy the hydrogen bonds in β-sheet structures, and GSH could reduce the internal disulfide bonds into sulfhydryl groups. The treatments with the protons, H2O2 molecules, hyperthermia, and GSH loosen the compact structures of SFNPs leading to the acceleration of drug release from these NPs. Many chemically active groups such as amino groups, carboxyl groups, and sulfhydryl groups, are present in the backbone of silk fibroin, and these groups are able to be

used for chemical modifications, which can endow SFNPs with some advanced functions (e.g., charge reversal, controlled drug release, and targeting property) [2].

Keiji et al. developed an ethanol-based method for preparing silk nanoparticles and then fabricated a biodegradable and biocompatible dual-drug release system based on silk nanoparticles and the molecular networks of silk hydrogels. Model drugs incorporated in the silk nanoparticles and silk hydrogels showed fast and constant release, respectively, indicating successful dual-drug release from silk hydrogel containing silk nanoparticles. The release behaviors achieved by this dual-drug release system suggest to be regulated by physical properties (e.g., β-sheet contents and size of the silk nanoparticles and network size of the silk hydrogels), which is an important advantage for biomedical applications. The present silk-based system for dual-drug release also demonstrated no significant cytotoxicity against human mesenchymal stem cells (hMSCs), and thus, this silk-based dual-drug release system has potential as a versatile and useful new platform of polymeric materials for various types of dual delivery of bioactive molecule [3].
Tomoda et al. (2021) produced a controlled-release device composed of SF microparticles embedded in SF hydrogel. First, they produced SF microparticles by an atomization method. Then dye molecules were incorporated into the microparticles by adsorption. The loaded microparticles were then mixed with regenerated SF solution. After treatment with 1–1 ethanol solution, gelation of the system was achieved. They studied the kinetics of dye release from the loaded microparticles and hydrogels containing loaded microparticles. It was found that the dye release from the microparticles reached equilibrium in approximately 90 min. Whereas for the hydrogels containing the loaded microparticles, the release persisted for 900 min, exhibiting a release 10 times longer. The dye release from the microparticles followed a Fickian diffusion mechanism. However, SF hydrogels containing SF microparticles followed an anomalous diffusion mechanism, showing that the release was correlated with the network degradation. Therefore, the used strategy holds the potential for developing prolonged release devices, especially for drugs and bioactive compounds [4].

Xia et al. adjusted the proportion of elastin in silk fibroin using genetic engineering technology and obtained silk-elastin like proteins (SELPs), which could form NPs via self-assembly. The first step was the spontaneous formation of micelles with silk blocks as the core structures, which was driven by hydrogen bonds among silk blocks; the second step was driven by the hydrophobic interactions among elastin blocks, leading to the orderly association of SELP molecules. During the assembly processes, drugs could be encapsulated in the SELP matrix to form NPs [2].

Exosomes represent a subtype of extracellular vehicles. They are formed by endosomes and are released by various cell types, to play roles in intercellular communication via autocrine, paracrine, and telocrine pathways [1]. A fibroin-based cryo-sponge was developed to provide controlled exosome release. Fibroin chains can self-assemble into silk I structures under ice-cold conditions when annealed above the glass transition temperature. Exosome release is enzyme-responsive, with rates primarily determined by enzymatic degradation of the scaffolds. In vivo experiments have demonstrated that exosomes remain in undigested sponge material for two months, superior to their retention in fibrin glue, a commonly used biomaterial in clinical practice. Fibroin cryo-sponges were implanted subcutaneously in nude mice. The exosome-containing sponge group exhibited better neovascularization and tissue ingrowth effects, demonstrating the efficacy of this exosome-encapsulating strategy by realizing sustained release and maintaining exosome bioactivity (Figure 4). These silk fibroin cryo-sponges containing exosomes provide a new platform for future studies of exosome therapy [5].

Conclusion:

The self-assembly of silk fibroin is a thermodynamic process which could be controlled by adjusting molecular mobility, charge, hydrophilic interactions and concentration. Based on these mechanisms, it is feasible to design silk fibroin materials with desired properties. Silk fibroin self-assembly consists of gradual conformational transition from random coil to β-sheet structure. Self-assembled silk fibroin hydrogels: can be used as bio interface materials and drug delivery systems. Advances in protein assembly preparation have provided new insights into long-term controlled drug delivery during tissue engineering.

References

1. Sun W, Gregory DA, Tomeh MA, Zhao X. Silk Fibroin as a Functional Biomaterial for Tissue Engineering. Int J Mol Sci. 2021; 22(3): 1499.
2. Ma Y, Canup BSB, Tong X, Dai F, Xiao B. Multi-Responsive Silk Fibroin-Based Nanoparticles for Drug Delivery. Front Chem. 2020; 8: 585077.
3. Numata K, Yamazaki S, Naga N. Biocompatible and biodegradable dual-drug release system based on silk hydrogel containing silk nanoparticles. Biomacromolecules, 2012; 13 (5): 1383-1389.
4. Tomoda BT, Pacheco MS, Abranches YB, Viganó J, Perrechil F, De Moraes MA. Assessing the influence of Dyes Physico-chemical properties on incorporaton and release kinetics in silk fibroin matrices. Polymes (Basel), 2021; 13 (5): 798.
5. Sun M, Li Q, Yu H, Cheng J, Wu N, Shi W, Zhao F, Shao Z, Meng Q, Chen H, Hu X, Ao Y. 1. Cryo-self-assembled silk fibroin sponge as a biodegradable platform for enzyme-responsive delivery of exosomes. Bioactive Materials 8 (2022) 505–514.