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REVIEW

A review on structure, preparation and applications of silk fibroin-based nano-drug delivery systems

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Abstract The development of highly effective systems for drug delivery is crucial in biomedical care. Protein-based carriers have gained more importance in drug delivery as they are more advantageous than conventional drug delivery systems. Silk fibroin is a naturally occurring protein derived from cocoons of Bombyx mori. It has high potential in biomedical fields for its excellent biocompatibility and biodegradability. Due to its unique properties, it is highly capable of loading and delivering various biomolecules, drugs and other moieties as therapeutics. Many emphases have been directed to develop SF-based nano-drug delivery for its strong binding capacity for a wide range of drugs, controlled drug release characteristics, and ease of fabrication. The recent developments on SF-based nanoparticles have been highlighted in this review, covering SF's chemical structure, properties and preparation methods. Also, recent functions of SF for the fabrication of nano-drug delivery systems are discussed.

Keywords Silk fibroin · Silk cocoons · Drug delivery · Nanoparticles · Cancer targeting

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Introduction

Drug delivery systems are composed of active drugs loaded in drug carriers. These drug carriers are crucial in developing drug delivery systems in the pharmaceutical industry. Therefore, research interests are significantly increasing in developing protein-based drug delivery platforms for their unique properties. These systems are biodegradable, non-antigenic, biocompatible, and exhibit various functional moieties that can initiate biological responses in cells [1]. Silk fibroin (SF) is a natural protein obtained from Bombyx mori cocoons. It has high potential in biomedical on its excellent biocompatibility and biodegradability. In the previous decade, research has witnessed plenty of strategies to formulate SF-based delivery systems for loading and releasing various drugs [2–4]. SF obtained from Bombyx mori has two chains linked by a disulfide bridge. One chain is heavy and is approximately 325 KDa and the other is light which is approximately 25 k Da. The heavy chain contains amorphous and crystalline domains [5]. The amorphous domain comprises bulky amino acids and the crystalline domain consists of glycine-alanine repeats connected with tyrosine and serine. The crystalline domains forming anti-parallel β-sheet structures are scattered among the flexible amorphous repeats. Silk cocoons consist of sericin (20%) and SF (80%). Sericin coating binds the two chains of SF together. Before processing fibroin, sericin must be removed from SF, which removes inflammatory and

thrombogenic responses. In addition, it has several properties such as high flexibility, strength, swelling characteristics, biocompatibility, biodegradability and non-carcinogenic, making it a unique polymer for the controlled or sustained delivery of drugs, enzymes, genes, and small molecules, in the fields of biomedical and biotechnology [5, 6].

SF, a protein-based macromolecule, has been employed in biomedical research as a biomaterial to fabricate nanoparticles (NPs), fibers, spheres, and hydrogels. SF-based NPs are particularly promising as a drug delivery strategy due to their biodegradability, high biocompatibility, cross-linking capability, and improved cell adhesion [7]. This review will discuss structural aspects and properties of SF and SF NPs, preparation techniques of SF-based NPs, and their applications as therapeutic drugs carriers. In addition, we have provided an overview of the recently reported methods for constructing SF-based drug delivery systems/ functions in drug delivery applications, with more focus on anti-cancer drug delivery.

Structure and properties

Natural silk obtained from silkworms at the macroscopic level comprises fibroin (70–80%), sericin (20–30%), a minor amount of fat/wax and ash. Fibroin is the major component of silk that serves as the inner core giving mechanical strength, while sericin serves as the exterior glue-like coating. It is been suggested that SF filaments are made up of nanofibrils. These nanofibrils combine to form microfibrils, which are bigger fibril units [8]. SF comprises polypeptide chains with molecular weights ranging from 200 to 350 kDa. SF's basic structure consists of repeating blocks of hydrophobic heavy chains (H-fibroin) and hydrophilic light chains (L-chain) with terminal C and N groups. Disulfide bonds serve as linkers between these chains. H-fibroin, L-fibroin, and P25 have a molar ratio of 6:6:1 in silk. H-fibroin's amino acid sequence can be described as (-Gly-Ser-Gly-Ala-Gly-Ala-)n [9, 10]. Repetitive hydrophobic domains of H-fibroin fold and link together to form anti-parallel β-sheet crystalline structures via hydrogen bonds, Van der Waals forces and hydrophobic interactions. Strong β-sheet interactions, a high degree of ordering, and a high density of β-crystallites are expected to absorb impact pressure and distribute it across the entire fibroin network, providing the silk network with outstanding mechanical strength. The secondary structure of silk can be divided into silk I, II, and III. Among these crystalline types, silk I is liquid, whereas silk II and III are solid. Silk I is a metastable form that is held in silkworm glands. It is described as a partially ordered structure having α-helix and random coil structures. On the other hand, silk II is formed after spinning that has a β-sheet crystalline structure. Finally, silk III has a trifold helical structure primarily [11–14]. The tensile strength of silk is greater than that of other biopolymers like collagen and polylactic acid, equivalent to nylon and mild steel despite its light weight [15, 16]. Furthermore, silk fibers have a better tenacity than Kevlar. As a result, silk has a very high strength-to-density ratio, making it ideal for excellent strength and low-density applications. The tremendous tensile strength and modulus of silk are due to the large volume of highly organized β-sheet crystalline structures. Attributing to its strong crystallinity and intrinsic hydrophobicity caused by a large number of intra and intermolecular hydrogen bonding, SF is insoluble in water and most organic solvents. However, aqueous SF solutions can still be obtained through regeneration. Furthermore, the thermal stability of SF film has been observed up to a temperature of 200 °C, at which the side chain amino acid residues break and the peptide bonds break down [12, 17–20].

Biocompatibility is one of the most essential characteristics of materials for biomedical applications. SF is a "clinically approved" biomaterial for human usage in numerous preclinical trials [21]. Biocompatibility is necessary to assure safety during application and administration; however, biodegradation is also essential for the administered biomaterial to be cleared entirely after service. The proteolytic degradation mechanism is responsible for silk's degradability, which may be precisely regulated by altering its processing parameters as well as its crystalline structure [22, 23]. Wang et al. demonstrated that SF scaffolds are biodegradable after a few weeks and also bioresorbable after one year from their in vivo study. Other synthetic polymers such as PGLA are degraded by bulk-hydrolysis, whereas SF is degraded by enzymatic surface erosion. When compared to other synthetic or natural polymers, SF offers distinct advantages for biomedical applications for its slower loss in

mechanical strength, inert by-products, and regulated degradation rate [24–27].

The regenerated liquid silk, a progressively deteriorated peptide combination of SF, might be created by dissolving silk fibers in concentrated neutral salts like CaCl2. Zhang Y Q et al. investigated SF NPs made quickly from liquid silk with water-miscible protonic and polar aprotonic chemical solvents [27]. The NPs are globular particles that are insoluble but well distributed and stable in an aqueous solution. According to Raman spectra, the tyrosine residues on the surface of the globules are more revealed than those on natural silk strands. The crystallinity of the silk NPs is around four-fifths that of the original fibers, according to X-ray diffraction. Their findings show that in an aqueous solution, the deteriorated peptide sequences of regenerated silk are collected homogeneously or heterogeneously to produce a looser globular structure. The looser globules of liquid silk are quickly distributed and instantaneously dehydrated internally and externally when exposed to high levels of organic solvent, culminating in more chain–chain contact, the alignment of those hydrophobic domains inside the globules, and the emergence of crystalline silk NPs with β-sheet configuration. The NPs' morphology and size depend on organic solvents' types, characteristics, and sometimes even molecular structures. More importantly, the looser globular substructure of degraded SF in an aqueous solution [28].

Utilizing innovative infrared spectroscopic methods, Carissimi G et al. investigated the secondary structure of SF during NP synthesis using ionic liquids and high-power ultrasound. SF fibers have 58% β-sheet, 9% turns, and 33% irregular and/or turn-like features, according to their infrared spectrum. When fibroin was dissolved in ionic liquids, its amide I band mirrored that of soluble silk, and there was no evidence of β-sheet absorption. SF NPs recovered from an ionic liquid solution contained an amide I band similar to silk fibers but with a lower β -sheet content and greater content of turns, implying an incomplete turn-to-sheet transition during the cycle. The amide I band form of SF NP closely approximated that of silk II from SF fibers after dissolution in ionic liquids and eventual regeneration into NPs, although with small differences that can be better shown from its second derivative. Different mechanisms, such as the use of polar chemical solvents, mechanical stress, and pH or ionic strength variations, can cause the creation of -sheets from silk I. Different regeneration techniques regenerate the β-sheet structures to varying degrees, as evidenced by the intensity disparity of their component bands. We infer that a similar sort of β-sheet structure is susceptible to the regeneration process in both forms of silk, and we indicate that it is structure A, based on the same spectral locations. In short, their SF NPs had 7% higher turn-like structures than SF fibers and a lower -sheet content as a result. Their findings show that I type A-sheet regeneration was incomplete, (ii) deformed and type B β-sheet structures are preferable over type A β-sheet structures in the approach used here, and (iii) turn-like structures transition into β-sheets [29–33].

Preparation of SF NPs

Figure 1 shows the extraction of SF from Bombyx mori cocoons. Initially, the cocoons are boiled in 0.02 M sodium carbonate for 30 min. This process is repeated thrice by replacing with fresh sodium carbonate solution each time. Next, the degummed silk fibers are rinsed with distilled water and excess water is squeezed. The fibers are allowed to dry at 50 °C in a hot air oven overnight. Extracted SF is then dissolved in Ajisawa's reagent ($CaCl_2$: ethanol: water in a molar ratio of 1: 2: 8) at 60 °C for 4 h. The dissolved SF solution is then dialyzed against ultrapure water for 48 h [34].

For the preparation of SF NPs from SF, there are various methods like salting out, desolvation electrospraying, microemulsion, and supercritical fluid technology [35–38]. Selecting the appropriate method for fabricating SF-based NPs is crucial for drug delivery, as these methods have their own advantages and disadvantages. SF has high molecular weight and protein composition, due to which NP preparation is challenging to manage. Furthermore, when SF is exposed to heat, pH change, salt, or severe shear, it tends to self-assemble into fibers or gels [39].

Salting out

This is a simple method for preparing protein-based NPs, where salting out of protein solution generates protein coacervates. Hydrophobic components of proteins interact with water and allow hydrogen

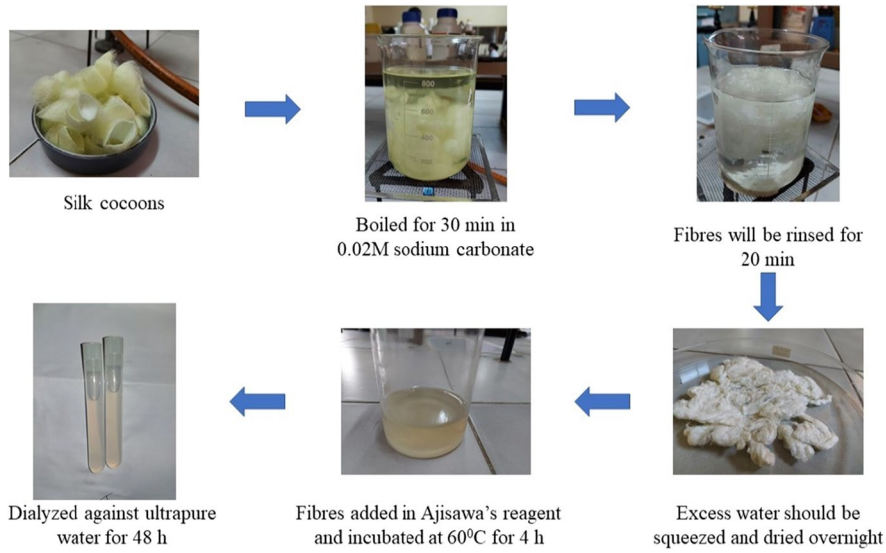

Fig. 1 SF extraction from cocoons

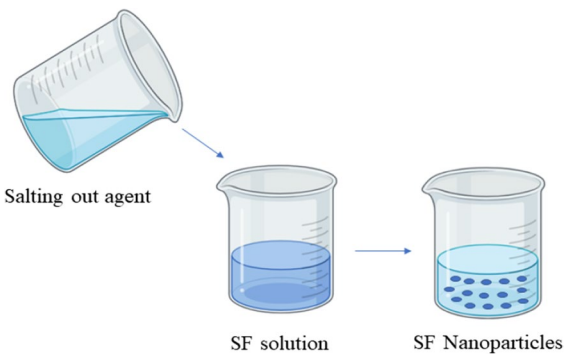

Fig. 2 Preparation of SF NPs by salting out method

bonds between proteins with water molecules in the surrounding. As salt concentration increases, certain water molecules are attracted to the salt ions, resulting in the removal of the water barrier between protein molecules and thus, protein–protein interactions increase (Fig. 2). As a result, the protein molecules develop hydrophobic contact with one another and precipitate out of the solution [40].

Desolvation

Due to relatively mild conditions, this approach is often used for creating protein-based NPs. Before being gradually extracted into a non-solvent phase, the protein is dissolved in a solvent. By phase separation, a phase containing colloidal coacervate and a second phase with solvent/non-solvent is generated (Fig. 3). The solvent must be miscible with the non-solvent in this process [41, 42].

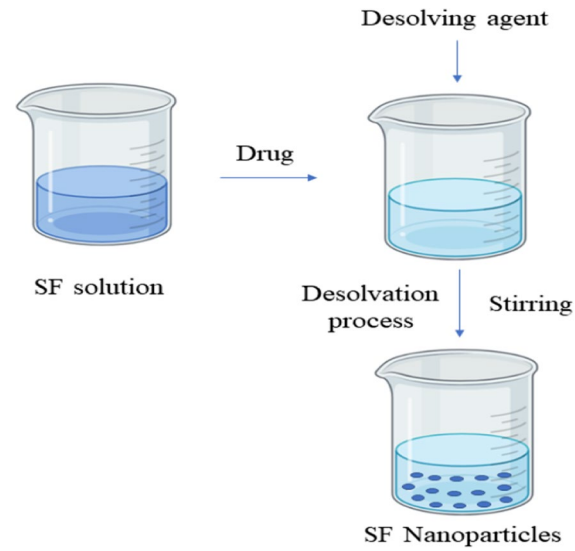

Fig. 3 Preparation of SF NPs by desolvation method

Electrospraying

This is an emerging approach for quick and high output generation of NPs where electrical forces to atomize liquids are used. The liquid flowing out of a capillary nozzle with high electric potential is driven

to scatter into minute droplets by the electric field in electrospraying (Fig. 4) [43, 44].

Microemulsion method

This method is based on surfactant-assisted thermodynamically stable dispersion of two immiscible liquids. The aqueous phase in w/o microemulsion generates nanosized droplets in a continuous phase which is hydrocarbon-based. Surfactant self-assembly is thermodynamically driven in this region, resulting in inverted or reverse micelles. The precipitate can be extracted by filtering or centrifuging the mixture by adding a solvent to the microemulsion, such as ethanol (Fig. 5). The main advantage of this approach is that it allows improved control on particle size where nature and surfactant concentration, cosurfactant, oil phase, and reacting conditions are altered [39, 45, 46].

Supercritical fluid technologies

In this approach, the solution consisting of supercritical CO_2 ($scCO_2$) and the solute is atomized by a coaxial nozzle that produces fine droplets. There is a nozzle having two coaxial passages. This allows $scCO_2$ and solution that is to be introduced into high-pressure vessel and temperature can be altered. The high velocity of $scCO_2$ breaks the solution into small droplets when the solution comes in contact with the $scCO_2$. Thereby enhancing mass transfer and mutual diffusion between supercritical fluids and the droplets. This results in phase separation and supersaturation of the polymer solution, nucleation and precipitation of polymer particles (Fig. 6). The $scCO_2$ acts antisolvent in the solution-enhanced dispersion by

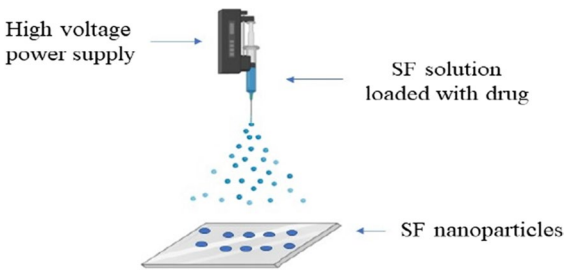

Fig. 4 Preparation of SF NPs by electrospraying method

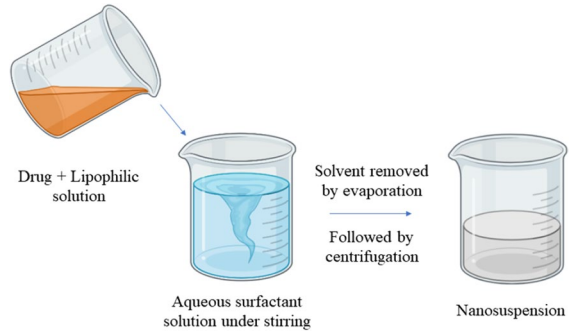

Fig. 5 Preparation of SF NPs by microemulsion method

supercritical fluid process (SEDS). The polymer's particle size distribution and morphology can be regulated by modifying SEDS process parameters like solution flow rate, solute concentration, temperature, and $scCO_2$ pressure [47, 48].

There are an expanding variety of SF-based systems that have been employed to encapsulate APIs and achieve modulated drug delivery, one of most explored of which are NP delivery systems, particularly for anti-cancer medicines. The charge and lipophilicity of such systems are determining variables for drug distribution and entrapment efficiency. Different medication release profiles emerge from changing these parameters. The salting-out approach is one of the most popular ways to make SF particles. Lammel et al., for example, used potassium phosphate as a salting-out agent to make SF particles with controlled diameters ranging from 500 nm to 2 m. The pH of the

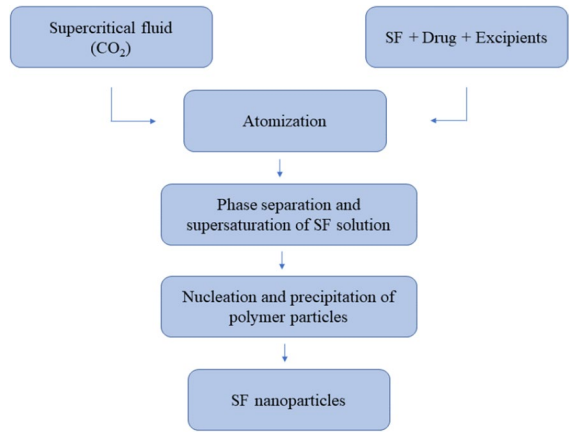

Fig. 6 Preparation of SF NPs by supercritical fluid technology

potassium phosphate solution influenced the SF particles' β-sheet structure and zeta potential. A far latest review on microfluidics used a desolvation process to synthesize smaller SF particles (150–300 nm) using a microfluidic setup (nano-assembler). The features of SF NPs were discovered to be regulated by two primary factors: flow rate and flow rate ratio. The use of microfluidic equipment allows the synthesis of SF NPs with desired sizes for drug delivery in a quick, repeatable, and regulated manner [35, 40, 49].

The microemulsion process was used by Myung SJ et al. to prepare NPs where surfactant, organic solvent, distilled water, and silk aqueous solution were mixed together to make the microemulsion solution. First, surfactant and cyclohexane were mixed together, then 400 l of silk aqueous solution were added and stirred for 1 min using a vortexer. The microemulsion was then broken up and the particles were recovered using a blend of methanol and ethanol. Next, the particles were dialyzed against ethanol for 24 h and then water for 48 h [36].

A high-pressure electrostatic generator and a micro-injection pump were used by Qu J et al. to make SFN. A high-voltage electric field was used to separate the SF solution into droplets. The resultant droplets were collected and frozen in a liquid nitrogen bath on a continuous basis. They were then freeze-dried using a lyophilizer. The lyophilized materials were suspended in deionized water and then placed in 1.5 ml centrifuging tubes to segregate nanoscale particles. To produce SF NPs, the tubes were centrifuged and the supernatant was freeze-dried again [37].

Zhao Z et al. used a high-pressure meter pump to deliver liquefied CO_2 to the high-pressure vessel in the SEDS method. The temperature of the downpour was kept under control. A steady supply of CO_2 was maintained until the appropriate pressure and temperature for keeping the CO_2 in a supercritical condition were achieved, and the system pressure was regulated by manipulating a downstream valve and tracked by a pressure gauge to maintain the pressure constant. The SF solution, which had been dissolved in HFIP, was then pumped into the high-pressure vessel via a stainless steel coaxial nozzle, along with supercritical CO_2, using an HPLC pump, and precipitation occurred. After the spraying was completed, the products were washed with fresh CO_2 for roughly 30 min to eliminate any remaining organic solvent from the precipitated particles. The CO_2 flow was halted after the washing procedure, and the pressure of CO_2 in the high-pressure vessel was gradually decreased to air pressure. After that, the SF NPs products were recovered on the filter and preserved in powder form in a desiccator at room temperature for characterization [38].

Table 1 represents the preparation methods used for fabricating SF-based nano delivery systems along with their applications.

Functions of SF in drug delivery

Silk proteins have successfully been used as drug delivery systems. These proteins are FDA-approved polymers. SF has plenty of unique properties that make it distinct from other natural and synthetic polymers in the field of drug delivery. Its main advantage is performing an aqueous process for loading sensitive drugs like proteins and nucleic acid. In addition, SF provides better resistance to thermal and enzymatic degradation of these therapeutics. This can be achieved by the conformational transition of α-helix and coiling highly crystalline β-sheets via mechanical stretching and ultrasonic treatment. Besides, SF is composed of amino acids containing several functional groups, including alcohols, thiols, carboxyl groups, and amines, that help in attaching sequences containing several functional groups, including alcohols and thiols carboxyl groups, and amines that help attach different moieties. The mechanism of binding biomolecules to SF is another crucial feature in controlled release drug kinetics. Electrostatic interactions are assumed to be the primary mechanism for drug loading and release on SF-based materials. The solid electrostatic interactions could avoid burst release compared to other polymeric-based carriers [23, 55, 65, 66].

The usage of NPs or particulate carriers reduces the delivery rate of solubilized drugs, in most cases, by introducing a second limiting step. Therefore, considering the properties of biomaterials is necessary, especially in terms of structure, composition, mechanical properties and functioning for fabrication of drug delivery systems. By altering the degree of SF functions per molecule, different drugs with different kinetics could be introduced into SF-based systems. This can provide a wide range of drug delivery platforms. This advantage of SF is significant when compared to other inert natural or synthetic polymeric materials. By genetic modification, it is possible to manipulate

Table 1 Methods for the preparation of various SF-based nano delivery systems

Sl. no	Type of delivery system	Formulation loaded with	Method of preparation	Application	Reference
1	SF-sodium alginate NPs loaded in hydrogel	Vancomycin	Injection method	For infected wound burn treatment	Rezaei F et al. [50]
2	SF nanofibers	Diclofenac	Electrospinning	Anti-inflammation	Opálková Šišková A et al. [51]
3	SF/HA scaffold	No drug-loaded in dressing	Freeze drying technique	Skin repair	Yang W et al. [52]
4	SF fibers	$CoFe_2O_4$ and Fe_3O_4	Electrospinning	Tissue engineering	Brito-Pereira R et al. [53]
5	SF microparticles	Cy7	Emulsion and dehydration technique	Intra-articular delivery	Mwangi T K et al. [54]
6	SF-coated liposomes	Ibuprofen	Ethanol injection method	Ocular drug delivery	Dong Y et al. [55]
7	Human serum albumin SF nanocapsules	Methotrexate	Ultrasound method	pH-responsive drug delivery	Claudia Tallian et al. [56]
8	SF NPs	Celecoxib and curcumin	Desolvation method	Osteoarthritis treatment	Barbara C et al. [57]
9	Inhalable SF microparticles	Ciprofloxacin	Spray drying	Non-cystic fibrosis bronchiectasis treatment	Liu C et al. [58]
10	SF hydrogel	Celecoxib	Ultrasound	Osteoarthritis treatment	Blasioli D J et al. [59]
11	SF NPs	Resveratrol	Nano-precipitation	Periodontitis	Giménez-Siurana A et al. [60]
12	SF hydrogel	Biliverdin and indocyanine green	Ultrasound	Wound healing	Yao Q et al. [61]
13	SF NPs	No drug-loaded	Antisolvent diffusion method	Transdermal delivery	Takeuchi I et al. [62]
14	SF NPs	5-Fluorouracil	Nano-precipitation	Controlled release delivery	Rahmani H et al. [63]
15	SF NPs	Indomethacin	Solution-enhanced dispersion by supercritical CO_2	Controlled drug delivery	Zhao Z et al. [64]

SF properties. SF sequence has these novel properties, which help in self-assembly to obtain the desired morphological characteristics. The sequence of the polypeptide endows new functionalities [67, 68].

SF has another prominent feature compared to other natural polymers; it can be a potential candidate as a lysosomotropic drug delivery system. It has the intrinsic ability to initiate the release of drugs in response to pH changes without chemical modifications [69]. Considering the clearance mechanism while using drug delivery biomaterials from the body is very necessary. The primary mechanism of clearance of specific biomaterials is absorption. By liver and kidneys, by-products of these materials could be cleared [70]. Proteolytic enzymes degrade SF. Hence, it is eliminated from the body, causing no side effects by absorption mechanism [66].

The SF coating is aimed at delivering drugs near the target cell. The major shortcoming of NPs is overthrown by this coating, where the nanosized moieties collide with the cell's surface with accurate vicinity and remain in place. Adhesion of SF coating to cells' outer layer of mucopolysaccharide enhances the attachment of the NPs to the cell surface when in contact with the cells [6]. Andrea S et al. studied specificity and targeting, by adhesiveness, of liposomes to keloid fibroblasts and human scar-producing cells. The kinetics, structural characteristics, and bioavailability of SF-coated emodin liposomal vesicles (SF-EL) were compared with uncoated EL. The organized

lamellar structure was observed in SF-EL. The coating also reduced the release rate of emodin. There was a change in kinetics during release due to swelling and the diffusional process. Targeting and adhesion were more for SF-EL against keloid fibroblasts. This might be due to the interaction between SF and the pericellular molecules surrounding the cells. Hence, SF coating improved the release kinetics and targeting of EL [6].

Applications of SF-based nano-drug delivery systems

Dissolving SF in high concentrations of neutral salts results in liquid SF which can be derived into various forms such as films, gel, powder, and fibers [5]. SF degradation is controlled by varying molecular weight or crystallinity, or cross-linking. SF-based systems have had significant attention for drug delivery in recent years. For example, Kaplan et al. worked on doxorubicin-loaded SF NPs, which act on stimulus-responsive anti-cancer nanomedicine to overcome drug resistance [69]. SF NPs could be loaded with drugs of therapeutic interest and exhibit pH-dependant in vitro release. Drug loading and distribution depend on charge and hydrophobicity in these SF-based systems, leading to different drug release characteristics [2]. The following are the areas in which various researchers have used SF-based nanosystems to achieve enhanced outcomes.

Small molecule delivery

Given the fact that the majority of pharmaceuticals on the market are small molecules, they nonetheless have several drawbacks, including hydrophobicity, limited permeability, short half-life, nonspecific targeting and distribution, and drug resistance soon after the first treatment. These difficulties can be efficiently addressed by NPs. NPs with a size range of 10–200 nm are commonly used to extend the period of the systemic circulation. NPs larger than 200 nm are mostly removed through the reticuloendothelial system, but NPs less than 10 nm may be cleared through the kidney. FNPs are increasingly being used as a delivery mechanism for various small compounds due to their versatility. However, as stated below, most research is focused on cancer treatments and anti-inflammation. As a result, other areas, particularly chronic illnesses, are wide open and desperately needed [71, 72].

Insulin delivery

Insulin delivery is the most crucial step for treating patients suffering from insulin-dependent diabetes mellitus. Researchers have tried almost all modes of delivery like oral, parenteral, and topical. However, within a short period, insulin undergoes hydrolyses to proteases and loses its activity [5]. Hai bo et al. reviewed that SF obtained from Bombyx mori is a biomolecular protein with excellent biocompatibility. Using glutaraldehyde as a linker, they prepared crystalline SF NPs on treating liquid SF with acetone and conjugated these NPs with insulin. This process improved the polypeptide stability in human serum and trypsin when analyzed in vitro. It is also easy to obtain purified insulin SF conjugates by repeated centrifugation process. Their results indicated that these insulin SF conjugates had a half-life of about two and a half folds more than the half-life of conventional insulin. Thus, SF has a latent interest in developing conjugate systems for enzymes of polypeptide delivery [5].

Ocular delivery

The eye has a unique structure and protective mechanisms. This reduces the bioavailability of ocular drugs. Improving the ocular drug efficacy can be achieved by the usage of mucoadhesive systems. Yixuan Dong et al. prepared liposomes coated with SF ocular delivery of drugs. Ibuprofen-loaded SF-coated liposomes (SFL) were prepared. These SFLs showed sustained release of the drug. By adjusting the concentration and protein chain of SF, permeation characteristics could be tuned. The conventional liposomes were positively charged when their zeta potential was examined. SF was negatively charged with an isoelectric point of 3.8–3.9, forming electrostatic interactions with liposomes. By the usage of cholesterol, SF coating onto liposomes may be increased [73]. On increasing SF concentration or temperature, fibroin chain interactions increased. This formed a high degree β-sheet structure. Drug release may vary due

to this [74]. SF has an excellent binding capacity to proteoglycans and glycoproteins. The attachment of SF to the mucopolysaccharide layer on the outer side of the cell could increase the attachment of SF drug delivery platforms to the cell surface. Their results indicated the efficiency and safety of SF as a biomaterial in ocular drug delivery systems. SFLs could be a promising approach in developing ophthalmic drug delivery platforms [55, 75, 76].

Gene delivery

SF has shown DNase resistance and good transfection efficiency in addition to biocompatibility. Because of these characteristics, SF is a preferred polymeric vector for gene delivery. Furthermore, SF can be genetically changed to acquire additional functionalities that are appropriate for the application. Although silk-based gene delivery vectors have demonstrated good transfection efficiency and a sufficient degree of specificity, better specificity is needed for more efficient targeted cancer therapy. F3 and Lyp1 peptides were added to recombinant silk proteins with a comparatively high amount of THP (25 mol percent, 3.4 kDa/13.6 kDa) to create an enhanced silk-based gene delivery method. F3 peptide attaches to MDA-MB-435 cancer cells particularly, whereas Lyp1 binds to tumor lymphatics selectively and can also induce apoptosis in MDA-MB-435 cells. The developed technique was able to deliver pDNA to tumorigenic cells with pinpoint accuracy. Recently, Song et al. have looked at how oligodeoxynucleotides (ODNs) are delivered to MDA-MB-231 breast cancer cells. The incorporation of SF to the NP formulation not only boosted cellular uptake of ODN by 70% but also lowered its cytotoxicity considerably. Due to their potential to penetrate or disrupt cellular membranes, cell-penetrating peptides (CCPs) have also been employed in SF-based gene delivery methods. CCPs are one of the chosen components to allow clathrin-dependent endocytosis of the particles when constructing a non-viral carrier for gene delivery [35, 77–82].

Anti-cancer drug delivery

Drug delivery to the tumor site has several barriers and requires careful attention while designing a new drug or new delivery system. The mode of delivery decides the drug efficacy and potency at the tumor site [6]. Drug delivery to tumor cells has significant challenges like damage caused to normal cells, low absorption and retention of the drugs at the tumor site [83].

NPs that are prepared using natural polymers, especially silk, have a prominent part in the delivery of drugs as these are highly biocompatible and biodegradable [84]. Hui Li et al. prepared SF-based NPs loaded with fluorouracil (FU) and curcumin. Curcumin has poor water solubility and cannot directly be used as aqueous solutions. However, SF NPs can carry and disperse drugs in aqueous solutions, increasing drug effectiveness in cancer treatment. Furthermore, Curcumin administration induces apoptosis upon generating ROS. This ROS could be a crucial pathway in tumor cell apoptosis induction, targeting mitochondrial and ER stress-related killing. Their results revealed that these SF NPs possessed a better anti-cancer effect when compared to the drugs in free form. Furthermore, SF NPs helped a more significant amount of drugs enter the tumor cells with controllable releasing ability. This might have induced apoptosis of cancer cells. By hematoxylin–eosin staining assay, it was found that more apoptotic cells were observed on treatment with these NPs. Animal studies suggest that tumors could significantly be reduced upon injection with these SF NPs loaded with 5-FU and curcumin. They concluded that these self-assembled polymeric systems would be an adaptable method in cancer treatment [83, 85, 86].

The most frequently used chemotherapeutic agent is cisplatin which has high antitumor activity. It is used to treat lung cancer, ovarian cancer and osteosarcoma. But this chemotherapeutic agent permeates through normal cells and spreads quickly. It binds to DNA and inhibits duplication of the DNA. This drug's other side effects include GIT discomfort, hepatic toxicity, and neurovirulence [87, 88]. Due to their high surface area and drug loading property, nanocarriers have been widely used to reduce the side effects of cisplatin and enhance its retention at the tumor site [89, 90]. By altering the degree of crystallinity, cross-linking and molecular weight of SF, its biodegradation rate can be regulated [91]. SF has carboxyl side chains of glutamic acid and asparaginic acid that offer ligand binding sites for drug loading. Qu J et al. fabricated SF-based NPs. They

prepared NPs loaded with cisplatin from SF solution using a high-voltage electrostatic field. Cisplatin was loaded into SF NPs via an interaction between the natural polymer SF and Cisplatin. Pt–Cl of Cisplatin interacted with the carboxyl groups of SF by ligand exchange, remarkably reducing the concentration of free $COO-$ groups. The bond between SF and Cisplatin was reversible due to its low nucleophilicity. The usage of these NPs could achieve sustained release of Cisplatin for up to 15 days. Cytotoxicity of these NPs was studied in vitro on A549 lung cancer cells and L929 mouse fibroblast cells, i.e., normal tissue cells. Cisplatin embedded in NPs released slowly and thus could not result in a sudden high concentration of free Cisplatin around normal cells. Cisplatin-loaded SF NPs induced apoptosis to cancer cells as these were easily internalized by adsorption endocytosis by A549 lung cancer cells. In contrast, these NPs showed poor cell growth inhibition in L929 mouse fibroblasts as they were not easily internalized. Thus, this work indicates that SF-based NPs exhibit sustained, specific inhibition of tumor cells [37]. All these properties of SF NPs make them promising drug delivery systems for therapeutic use in lung and other cancers.

SF has hydrophobic domains, consisting of antiparallel β-sheet layers. It can be purified upon treatment with boiling sodium carbonate solution to remove sericin. This sericin-free SF has noticeable applications for its low inflammatory response and adjustable biological responses [92, 93]. SF is more exploited in biomedical areas for its novel aspects in processing and properties like elasticity and strength [94]. The tumor microenvironment has cells, signalling moieties and an extracellular matrix surrounded by blood vessels [95]. Around 85% of most tumor-type tissues are cancers in epithelial tissue. Since the significant functional tissues like the kidneys and liver consist of fenestrated endothelium, more attention must be paid while developing nanocarriers with proper charge and size targeting these cancer cells [96]. Ming Hui Yang et al. studied the uptake of SF-chitosan NPs (SF-CN) by liver cells. These SF-CN have been more significantly focused as carriers, of chemotherapeutics, for their stability, ease of preparation and versatile administration routes. Through enhanced permeability retention effect, these NPs target the tumor cells. In cells, these SF-CN accumulate around the affected area by hydrophobic interactions. The usage of SF-based derivatives for biomedical applications was evaluated. SF-CN was further modified with SF to attain better cellular responses and HepG2 cellular uptake was observed. They found that these SF-CN may be non-toxic to HepG2 cells from proteomic approaches. The CSNPs and SF-CSNPs were non-toxic, unlike the metal-based or metal oxide NPs. They also provided a new method for detecting proteins in assessing hepatic cells' response to a polymeric system. Although they could not justify the significance between SF-CN and cell mitosis process, these nanosystems could be considered anti-cancer delivery agents [92]. Figure 7 indicates the delivery of anti-cancer drugs via SF-based systems.

Functionalization of SF NPs for cancer targeting

SF NPs have a higher surface area to volume ratio than larger particles, which is one of its primary strengths. Furthermore, when targeting cancer cells, the loaded APIs must be delivered at the desired concentration in order to achieve a desirable effect on the cancer cells while causing minimal damage to other cells [97]. As previously stated, SF has several active amino groups that can be employed to bind to other macromolecules [98]. SF has been functionalized with biological recognition domains such as the RGD sequence, folate, and other biological molecules for tissue-specific drug delivery. Furthermore, SF has been applied to coat the surfaces of polymeric microparticles and liposomes to boost their cell adherence [34]. The Arg-Gly-Asp (RGD) sequence, which acts as a ligand for cell surface integrin receptors, can be coupled to SF particles to improve their adhesion to cancer cells that overexpress integrins; for example, Modifying the surface of silk NPs with folate could be employed as a tumor-targeting approach in a similar manner [99]. The addition of folate to silk particles improved not just the NPs' retention at the tumor

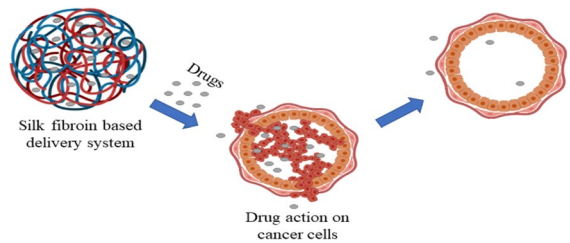

Fig. 7 SF-based delivery system for anti-cancer drug delivery

Table 2 Functionalized SF NPs targeting cancer

Sl. no	Drug used	Nanoparticle type	Functionalization	Cancer cell lines used	Reference
1	Doxorubicin	Gold NPs	SF	HeLa	Horo H et al. [101]
2	Gemcitabine	SF NPs	SP5-52 peptide	LL-2	Fatemeh Mottaghitalab et al. [102]
3	Doxorubicin	SF NPs	Radiolabelling with Technetium-99 and tween 80	C-6 and LN-229	Pandey V et al. [103]
4	Au–Ag	SF NPs	Cellulose acetate	MCF-7	Arumugam M et al. [104]
5	Quercetin	SF NPs	Lyp-1	4 T1	X Zhang et al. [105]
6	Curcumin	SF NPs	Chondroitin sulfate	264.7 macrophages	S Gou et al. [106]
7	DOX	SF NPs	Amorphous Calcium chloride	4 T1	Tan M et al. [107]
8	Curcumin	SF NPs	Arg-Gly-Asp Cyclopentapeptide	CACO-2 and HeLa	Bari E et al. [108]
9	-	SF NPs	Functionalized with molecularly imprinted polymer (MIP)	LDH and NIH 3T3	Bossi AM et al. [109]
10	Doxorubicin	SF NPs	Folic acid	HeLa	Ning Sun et al. [110]
11	Doxorubicin	Silk spheres	Her2 binding peptide	D2F2E2/LUC	Florczak A et al. [111]
12	Cisplatin	Silk-based particles	Genipin	A549	Kim SY et al. [112]

location but also their cellular uptake [100]. Table 2 shows the various SF NPs which are functionalized with certain biomolecules for targeting cancer.

Outlook

SF is a unique polymer with a wide range of applications such as designing, engineering, and administration in drug delivery systems for its wide range of mechanical qualities, molecular structures, morphology, and flexibility. In various biomaterials requirements, SF's slower decomposition rate in vivo with adjustable control on organization, shape, and chemical properties proposes a series of standards for protein-polymer relationships in biomaterials requirements. The rationales of SF-based NPs are efficiently organized. These are capable of controlling the release rate of biomolecules uninterruptedly and constantly.

Conclusion

Improving the efficacy and reducing off-target effects of therapeutics in biomedical research has been the primary aim of scientists. Various drug delivery approaches have been and are also under development for this purpose. However, few SF-based systems have been reported to overcome the side effects of conventional delivery methods. As discussed above, SF has versatile properties that make it a potential compound in biomedical research. We have discussed the various techniques and strategies adopted by researchers involving SF for improving therapeutic efficacy and recent methods for generating functionalized SF for targeted drug delivery. In cancer research, it has shown better cytotoxic effects to the tumors, specifically, without affecting the normal cells. Thus, SF-based systems could provide novel strategies and opportunities for enhanced outcomes. The potential prospect of SF for biomedical industries is currently being raised.

and becoming a reality as companies with commercialized products and continuous clinical trials develop. We believe that the new Silk Road might connect the old textile industry with future healthcare applications.

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Declarations

Conflict of interest The authors declare no competing interests.

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