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REVIEW ARTICLE OPEN ACCESS

Silk Proteins in Drug Delivery: An Overview

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Abstract

Primarily Silk is classified as Mulberry silk (collected from *Bombyx mori*) and Non-Mulberry silk (collected from sources other than *Bombyx mori)*. Whilst Mulberry silk has gained its importance in biomedical application due to superior biocompatibility and biodegradable properties when compared to synthetic protologues; such edge cutting popularity is quite new among Non-Mulberry variant. Silk proteins namely Sericin and Fibroin, are reported to have been employed in tissue engineering and drug delivery owing to its biocompatibility, slow biodegradability, self-assembly, excellent mechanical properties and controllable structure and morphology. Silk is less inflammatory than other common biodegradable polymers. Fibroin is the fibre used in textile and biomedical devices whereas Sericin is glue like material which binds the fibres together. The fibroin is further divided into two, based on the molecular weights of chains of amino acid. Sericin, being the glue-like material and constitute the part of silk which was generally washed away during extraction of fibroin used as textile material. Researchers have reported that Sericin do not produce immunogenic responses unless associated with fibroin. The review focuses on silk proteins and its utility in drug delivery.

Keywords: Sericin, Fibroin, *Bombyx mori*.

INTRODUCTION

Silk is a well known textile material, discovered by Emperor Huang Ti's 14 year-old bride, Hsi Ling Shi, while she was sipping her tea, according to Chinese folklore. It eventually became a valued textile commodity across the globe. The discovery of silk in ancient Chinese history was breakthrough in the field of unnamed textile technology because of its elegance and had many historical events associated with it. The "Silk route" being named after the trading of silk from China to Mediterrean region through India which also led to the secret of sericulture spreading across the distance covered by traders; according to Chinese popular folklore.

The popularity of silk as valued textile material was not constrained but as biopolymer too[1-3] which is evident from increasing publications regarding silk and it's proteins. Silk may also be considered as a natural protein fibre, consisting of repeating sequence of amino acid.[4] The two different parts of silk protein include fibroin and sericin. These silk protein molecules have properties, offering utility for drug and gene delivery. Silk is employed in making of biomedical sutures for ages and has achieved Food and Drug Administration (FDA) approval for biomaterials device utility.[2]

Types of Silkworms

The silkworms were classified as mulberry and Non-mulberry. *Bombyx mori* L is Mulberry silk is easily domesticated.[3] The Non- mulberry silkworms, are wild heterogeneous in nature These silkworms can further be broadly classified into two major categories according to their habitat: temperate and tropical. The different types of silks available are mentioned in Table 1 below.

Table 1: Different types of silk and it's biological and geographical source

S No.	Name	Biological Source	Geographical Source
1	Mulberry silk	Obtained from the silkworm, which feeds on the leaves of mulberry plant and is known as *Bombyx mori*	In India, the major mulberry silk producing states are Karnataka, Andhra Pradesh, West Bengal, Tamil Nadu and Jammu &Kashmir
2	Tasar	Obtained from silkworm which feeds on Asan and Arjun. The Biological Name for the silkworm is *Antheraea mylitta*	Produced in the states of Jharkhand, Chattisgarh and Orissa, besides Maharashtra, West Bengal and Andhra Pradesh in India.

3	Oak Tasar	It is finest quality of Tasar obtained from the silkworm *Antheraea mylitta* , feeding on oak trees	The silkworm is found in abundance in the sub-Himalayan belt of India including the states of Manipur, Himachal Pradesh, Uttar Pradesh, Assam, Meghalaya and Jammu & Kashmir. China is the major producer of oak tasar in the world
4	Eri	Eri silk is a product of the domesticated silkworm, which feeds mainly on castor leaves and therefore it got it's name *Philosamia ricini*.	In India, mainly in the north-eastern states and Assam. It is also found in Bihar, West Bengal and Orissa.
5	Muga	It is obtained from silkworm, *Antheraea assamensis.* These silkworms feed on the aromatic leaves of Som and Soalu plants and are reared on trees similar to that of tasar.	Found in Assam

Note: Silk fibroins have been used for ages as textile material. The quality of silk depended on the species of silkworm from which the fibroin was collected; after removing the unwanted glue-like material called sericin. The rearing of silkworm depended on the type of vegetation in particular locality and helped in economical growth in that region. The above table is created from the data available from Central Silk Board, India http://krishikosh.egranth.ac.in and http://www.csb.gov.in/silk-sericulture/silk.

Silk Protein

Silk proteins are namely Sericin and Fibroin. Fibroin is the part of silk cocoon which contributes to the silk toughness while Sericin helps in formation of the cocoon and constitutes about 25–30% (in case of *B. mori*) of the total cocoon weight. Cocoon construction of non-mulberry silkworms differs from cocoons of both mulberry and other members of the same family of non-mulberry silkworms. The construction of cocoon differs from mulberry to non-mulberry. The cocoon of *B. mori* possess 10 layers while tropical tasar composed of three layers only. The Sericin content is variable and thus recombinant variants are used for utility in various purposes. [5-10]

Silk fibroins

Silkworm fibroin is used as biomedical sutures from ages, it is also used in textile production for clothing as the sericulture process is quite easier than spider silk.[11-14] The silk fibroin from the cocoon of silkworm *Bombyx mori* silk proteins, contain two major components, light (~25 kDa) and heavy chain (~325 kDa) fibroins.[15] The core sequence in the heavy chain is reported to include alanine-glycine repeats. In silkworm cocoons, these two fibroins are enclosed in a sericin coat, glue-like proteins, to form the composite fibers of the cocoon.[16,17] The Beta Pleated structure of silk fibroin helps in controlled release of drug.[17]

Silk Sericin

Silk fibroin's use as textile material is quite old and has found its utility in medical applications as well.[5,18,19] The extraction of fibroin during degumming process involves washing away of sericin protein. The lost sericin protein also posses threat to environment as it is held responsible for increase in biological oxygen demand during decomposition. Sericin is a polypeptides consisting of 17–18 types of amino acids among which serine, aspartic acid, and glycine are the three most abundant amino acids.[6]

Sericin has been categorized into A, B and C, based on its solubility in water. The outermost layer is Sericin A and is most soluble. The intermediate part is Sericin B similar to Sericin A but solubility is less in water. The innermost layer is Sericin C which is insoluble in hot water. [15]

Sericin is reported to have properties such as anti-oxidation, anti-bacterium, anti-coagulation and promoting cell growth and differentiation. Sericin is reported to have properties to become hydrogel with sustained release property, pH dependent degradation dynamics and good elasticity. The sericin is also reported to form injectable hydrogel when cross-linked with Glutaraldehyde. The extraction of sericin is shown to have effect on desired properties as harsh chemical have denaturing effect on amino acids. The use of Lithium Bromide in degumming process is most suitable for hydrogel formation as gelling process is faster. [16-21]

Techniques for Preparing Silk-Based Drug Delivery Devices

Oral drug delivery System

Theophylline demands to be released at controlled rate and thus silk fibroin along with Polyethylene Glycol (PEG) and silk fibroin with 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) is used as coating material. The result have differed for PEG as it exhibited brittleness but with EDC have shown to be releasing drug at zero order. [22]

Gelation

Sericin was reported to have no immunogenic responses unless associated with silk fibroin the quantity of silk sericin is also less as it constitutes about only 20-30% of silk cocoon, the sericin part of silk was also used in preparation of hydrogel. The natural fibroin deficient mutant silkworm was used. The silk servicing was cross linked with glutaraldehyde and fabricated into molded structure, injectable and hydrogel. [24]

Vortex induced hydrogelation in silk fibroin was reported and increasing the vortex time increased the solution turbidity and eventually bulk phase and solid like material separation was observed, particularly at lower protein concentrations. Both circular dichrosim and rheology data were collected from turbid solutions after removal of the solid phase.[25]

Microparticle

The fabrication of silk fibroin spheres or microparticles which can be carried out under mild conditions, using aqueous silk fibroin solution under room temperature which can be used to encapsulate temperature sensitive materials.[26] The spheres can be prepared by a laminar jet break-up induced by a nozzle vibrating at controlled frequency and amplitude. The size and morphology of the spheres may be tuned by varying the diameter of the nozzle, the concentration of silk fibroin solution and the final treatment to induce water insolubility such as using organic solvents.[27]

Silk Sericin has found its utility in tissue engineering[23] and drug delivery as well. Alginate microsphere containing silk sericin was prepared using electrospraying and showed controlled drug delivery with high encapsulation efficiency. Electrospraying is a process of liquid atomization by means of electrical forces. The process includes the high voltage is application to a liquid flowing out of a capillary nozzle. The liquid is expelled by the electric field to be dispersed into minute droplets.

Freeze-drying

Freeze-drying is a mild fabrication process and leads to porous structures which largely affect drug release kinetics.[28-32]. Exposure to freezing temperatures prior to Lyophilization has a significant effect on the pore size of silk fibroin matrices. The appropriate blending of silk fibroin with another polymer proved to be of better tissue engineering product. Lowering the temperature resulted in smaller, highly interconnected pores and high porosity, whereas higher freezing temperature led to larger pores and lower porosity. Moreover, higher silk fibroin concentration resulted in smaller pores and less inter-connectivity.[33]

Electrospinning

Electrospining process is used to form nanowoven mass using strong electrical field. The electrical field is applied between a grounded target and a polymer solution that is pumped from storage chamber through a capillary orifice. A jet is produced, when the voltage reaches critical value and charge overcomes the surface tension of deformed drop of polymer solution formed at the capillary orifice. Further evaporation of solvent helps in the formation of nanowoven fibre. Factors like voltage viscosity, composition and feeding rate of the polymer solution, can be fabricated to get desired diameter of fibre and porosity of fibre. The large surface area of the nanowoven fibre can easily be degraded and can release drug at desired rate. For drug loading, aqueous silk fibroin solutions may be directly loaded with drugs, either by dissolution or colloidal dispersion, and then electrospun.[34]

Layer-by-Layer Deposition

The primary driving force for the surface assembly of silk fibroin is by hydrophobic interactions while electrostatic interactions play a secondary role. The possibility to coat hydrophobic and hydrophilic materials, and the possibility to control the composition and the thickness of the films by the number of deposited silk fibroin layers, silk fibroin concentration, salt concentration in the dipping solution and rinsing method makes the layer-by-layer deposition of silk fibroin attractive for controlled drug release application. Drug molecules may be incorporated either in or between layers.[35-38]

Preparation of Silk Nanoparticle

Desolvation

The desolvation/coacervation process is the process in which it reduces the solubility of the protein leading to phase separation. The addition of desolvating agent leads to conformation changes in protein structure resulting in coacervation or precipitation of the protein.

The preparation of the silk fibroin nanoparticles can be done by mixing the aqueous SF and water miscible protonic organic solvents (methanol, ethanol, propanol and isopropanol) or polar aprotonicorganic solvents (tetrahydrofurnan and acetone). The desolvation was also reported to carry out using dimethyl sulfoxide (DMSO). The altered process consisted of the following steps namely, protein isolation, desolvation, centrifugation, purification, sonication, filtration and Lyophilization. The desolvation process was further fabricated by adding a small amount of ethanol in regenerated silk fibroin solution and quenching the mixture below freezing point.[40]

Capillary-Microdot Technique

Gupta *et al* prepared Silk fibroin (SF) -encapsulated curcumin nanoparticles less than 100 nm in size using the devised capillary-microdot technique. In brief, curcumin was added into SF solution to form drug suspension. Then the suspension was dispensed on glass slides via a microcapillary. The slides were then frozen overnight and lyophilized. The resulting dry dots containing SF-encapsulated curcumin nanoparticles were scraped off the slides and were crystallized by methanol treatment. The nanoparticles collected by centrifugation.[41,42]

Salting out

A simple approach for preparation of protein-based nanoparticles is the salting out of a protein solution to form protein coacervates. Proteins have hydrophilic and hydrophobic parts. Hydrophobic parts can interact with the water molecules and allow proteins to form hydrogen bonds with the surrounding water molecules. With the increase of the salt concentration, the salt ions attract some of the water molecules, resulting in the removal of the water barrier between protein molecules and the increase of the protein-protein interactions. Therefore, the protein molecules aggregate together by forming hydrophobic interactions with each other and precipitate from the solution.

The salting out process was reported to be carried out using potassium phosphate (>0.75 m) and eventually collected by centrifugation. The use of higher concentration of potassium phosphate resulted in formation of larger particles. [43]

Supercritical Fluid Technologies

Silk fibroin nanoparticles with a particle size of about 50 nm via solution-enhanced dispersion by Supercritical Carbon Dioxide (SEDS) for the first time successfully. The influence of process parameters on particle size and SF nanoparticles formation mechanism were investigated. The results indicated that precipitation temperature, concentration and flow rate of SF solution have a positive effect, while precipitation pressure has a negative effect. The nanoparticle formation mechanism was elucidated with the formation and growth of SF nuclei in the gaseous miscible phase evolved from initial droplets generated by the liquid-liquid phase split.[43,44]

Electrospraying

Silk fibroin (SF) nanoparticles was prepared with a uniform spherical shape and an average particle size as low as 80 nm by the electrospraying technique. Increasing the concentration of SF solutions, feed rate and needle-collector distance increased the average particle size of the SF nanoparticles. Increasing voltage decreased the particle size up to 20 kV, but with higher voltages (25 and 30 kV) the average particle size increases. The resulting SF nanoparticles exhibited a β-sheet structure, similar to fibroin filaments but with a lower

crystallinity index. No functional group change occured in the process of electrospraying. [45]

Electric Fields

Leisk *et al.* reported an electrically mediated hydrogel (e-gel) from SF. The e-gel samples that were freeze-dried at −80°C exhibited extended and spherical, micellar, micrometer-scale structures. Lu Q *et al.*found the formation of the SF nanoparticles with sizes of tens of nanometers was a critical step in the formation of e-gels. Under an electric field the nanoparticles aggregated to form nano- or microspheres on the positive electrodes owing to screening of the negative surface charge, which could otherwise prevent intermolecular self-assembly of SF in neutral solution.[46,47]

Future Scope and Conclusion

The silk proteins have unlimited scope for delivering drug at controlled rate. The use of silk proteins in Tissue Enginnering is also remarkable. The beta pleated structure induces gelation which can be considered as stability hampering factor as shipping of finished product. Thus future researches might focus on silk based product stability.

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