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Fibroin Nanoparticles: Use in Drug Delivery

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

The silkworm *Bombyx mori.*L. is infected by various diseases viz; grasserie, flacherie, muscardine, and pebrine. Among all these diseases the grasserie causes major economic loss to the industry and is one of the main reasons for low silk productivity. It is caused by a virus known as *Bombyx mori* nucleopolyhedro virus (BmNPV). The impact of grasserie disease on silkworms is significant as it leads to reduced silk production and can result in economic losses for sericulture farmers. In India greater than 50% of silk cocoon crop loss is due to BmNPV [1] and in Kashmir valley, the loss is about 28-32% [2]. Silk obtained from the cocoons of silkworm *Bombyx mori* L. is a natural fibrous protein well known for being lightweight, having high mechanical strength, good flexibility, and luster making it ideal for the textile industry. In addition, fibroin extracted from the cocoons of domesticated silkworm *Bombyx mori* L. has gained growingly interest due to its excellent

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mechanical properties and high biocompatibility, biodegradability, inexpensiveness, and preparation flexibility [3]. These properties of silk fibroin lead to the formulation of fibroin nanoparticles (FNP'-s) which can be used to encapsulate different types of therapeutic compounds like proteins, vaccines, enzymes, etc. Fibroin has been approved as a biomaterial by the Food and Drug Administration (FDA) and has been popularly used in numerous medical applications such as sutures, tissue regeneration, coating devices, and drug delivery systems [4]. It has been studied that SF-derived curcumin nanoparticles show higher efficacy against breast cancer cells and have the potential to treat in vivo breast tumors by local, sustained, and long-term therapeutic delivery as a biodegradable system [5]. Therefore, fibroin-coated nanoparticles can be used effectively for disease management.

Keywords: Bombyx mori; drug delivery; fibroin; nano-particles

1. INTRODUCTION

Many drug delivery systems have been developed recently to administer multiple drugs and release them in a controlled manner to maximize the efficacy of therapies [6]**.** Over the past few decades, the development of drug delivery systems (DDS) has been fueled by the design and synthesis of numerous biocompatible materials [7]. A drug delivery system refers to the method of administering therapeutic agents to the body in a targeted and controlled manner. The goal is to ensure that the drug reaches the intended site of action, in an appropriate concentration and at the right time. A drug delivery system is made up of a drug carrier that the active ingredient is adsorbed or attached to, or in which the active drug is dissolved, dispersed, or encapsulated [8]. Other highly appealing techniques that have received a lot of attention include targeted delivery, slow delivery, and controlled rate drug delivery [9]**.** Using nanoparticles is one method of delivering medication to the brain. Polymeric particles known as nanoparticless are composed of synthetic or natural polymers and range in size from 1 mm to approximately 1000 mm. Drugs can be chemically bonded, adsorbed to the surface, or bound within a solid solution or dispersion. To date, only poly (butyl-cyanoacrylate) nanoparticles have proven effective in the in vivo administration of pharmaceuticals to the brain. The first medication administered via nanoparticles to the brain was hexa-peptidedalargin (Tyr-D-Ala- Gly- Phe-Leu-Arg), an opioid-active analog of leu-enkephalin [10]**.**

With their additional ability to combine therapy and diagnosis, nanoparticles offer significant advantages in drug delivery, release, and targeting. As a result, they have become a key tool in nanomedicine. Enhancing their stability in a biological setting, mediating the bio-distribution of active compounds, and enhancing drug loading, targeting, transport, release, and interaction with biological barriers are the primary objectives [11]. While there are many effective drug delivery systems in use today, there are still some issues that need to be resolved and cutting-edge technology needs to be created in order to successfully deliver medications to their intended locations. Therefore, research is currently being done on nano-based drug delivery systems, which will enable more sophisticated drug delivery systems [12].

It is anticipated that the medications will be able to effectively target the disease-causing cells at a precise therapeutic concentration. On the other hand, it is observed that the release rate, stability, and ability to target specific cells and tissues are uncontrollable and cannot be observed [13]. Therefore the drug delivery system is made to overcome these obstacles [14].

One innovative approach to drug delivery involves the use of fibroin nanoparticles. Fibroin is a protein found in silk that possesses unique properties suitable for drug delivery. Silk fibroin (SF) is a naturally occurring protein polymer with several unique properties that make it a suitable material for incorporation into a variety of drug delivery vehicles capable of delivering a range of therapeutic agents. SF is a crystalline protein-based fiber that forms the structure of silk fibers, and it is composed of 65-75% fibroin, along with other components such as sericin, wax, pigments, sugars, and impurities. It has been demonstrated that SF matrices can effectively deliver biomolecules, small molecules, and anticancer medications [15].

Drugs can be encapsulated in fibroin nanoparticles and delivered to particular body tissues or cells. Targeted delivery, controlled release, and enhanced drug stability are just a

few benefits of this encapsulation. Fibroin nanoparticles' biocompatibility their ability to be well-tolerated by the body and their low risk of adverse reactions is one of their main advantages. This makes them a desirable alternative for delivering a variety of medicinal substances, such as drugs that could be susceptible to deterioration or have unfavorable side effects when taken as prescribed. Fibroin nanoparticle surface characteristics can be changed to enable targeted delivery to particular tissues or cells. By doing so, the medication's exposure to healthy tissues can be minimized, potentially lowering side effects and enhancing the overall safety of treatment [16]**.** In many different biomedical applications, including films, hydrogels, spheres, three-dimensional scaffolds, and electrospun fibers, SF-based nanoparticles have been widely employed. SF-based nanoparticles are suitable for targeted and long-term drug delivery because they can release drugs under controlled circumstances [8]**.** Silk fibroin has low immunogenicity, good biocompatibility, and biodegradability [17]**.** Hence making a silk fibroin as an appropriate measure for the drug delivery system.

2. AIM OF THE REVIEW

This review article has been written to accumulate the research being done on silk fibroin nanoparticles as a suitable drug delivery method. This review article covers the biochemistry of fibroin nanoparticle, the use of fibroin as a nanoparticle, the analysis of fibroin as a novel nanoparticle for drug delivery systems, synthesis and extraction, assays to ascertain the properties of fibroin nanoparticles, strengths and limitations of fibroin nanoparticle as a drug delivery system, and future outlook.

3. BIOCHEMISTRY OF FIBROIN NANOPARTICLE

The silkworm, Bombyx mori L., secretes a naturally occurring protein fiber known as silk. Silk is used by the insect to shield their pupa while they undergo the process of metamorphosis into moths [18]**.** The protein extracted from silk fiber, known as domesticated *Bombyx mori* silk fibroin, has been used as a promising biomaterial in recent years for use in tissue engineering, drug delivery, and other biomedical applications [19]**.** Fibroin nanoparticle biochemistry includes the synthesis and characterization of these nanoparticles for use in drug delivery systems [20]**.** SF is a bio-macromolecule that is based on proteins and consists of large, repetitive, modular hydrophobic -domains that are broken up by tiny hydrophilic groups. Fibroin nanoparticle biochemistry includes the synthesis and characterization of these nanoparticles for use in drug delivery systems [8]**.** The main components of *Bombyx mori* SF's primary structure are serine (Ser) (12%), alanine (Ala) (30%), and glycine (Gly) (43%) [21] and tyrosine (Tyr) (5%) also. SF is a heterodimeric protein consisting of a heavy (H) chain (~325 kDa) and a light (L) chain (~25 kDa) connected by a single disulfide bond at cys-172 of the L-chain and cys c-20 of the H chain [22,23]**.** Moreover, P25, a 25 kDa silk glycoprotein, is connected to heavy and light chains by disulfide bonds through noncovalent interaction furthermore having a 6:6:1 molar relation [24]**.** Both hydrophobic and hydrophilic blocks can be found in the amphiphilic heavy chain of SF. By folding into β-sheets, the hydrophobic blocks' repeating sequence of Gly-Ala-Gly-Ala-Gly-Ser is what gives SF its crystalline structure. But compared to the hydrophobic region, the hydrophilic region is a shorter, non-repetitive segment [25]**.** Fibroin is therefore insoluble in water and may be regarded as a hydrophobic glycoprotein [26]. In general, fibroin nanoparticle biochemistry entails processing and modifying silk fibroin to produce nanoparticles with desired characteristics and investigating how these nanoparticles interact with biological systems for a range of biomedical applications [27]**.**

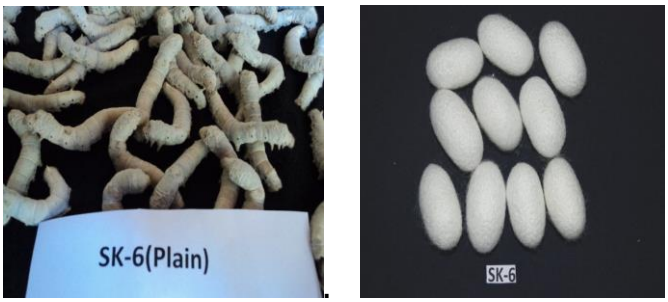

Fig. 1. Source of Fibroin Protein

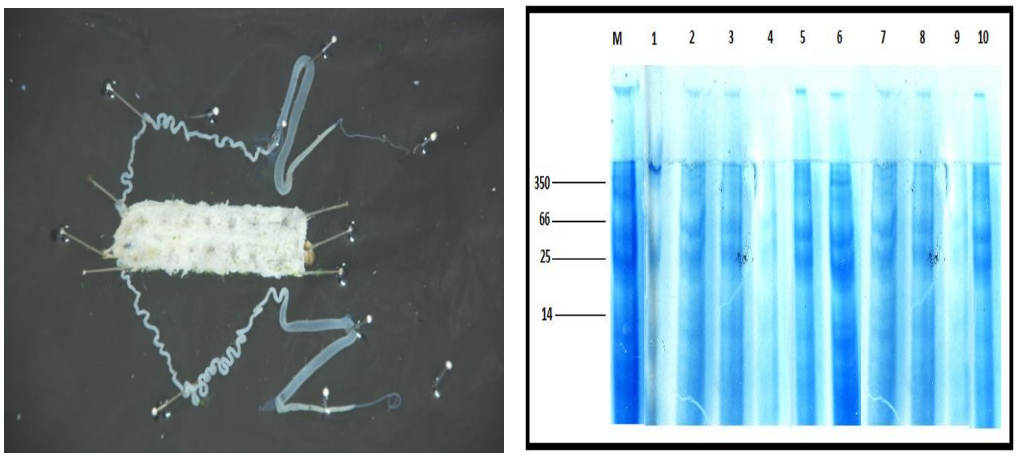

Fig 2. Isolation & Purification of Silk Protein

4. FIBROIN AS A NOVEL NANOPARTICLE FOR DRUG DELIVERY SYSTEM

Fibroin, a natural polymer, has been investigated as a potential material for drug delivery systems. For controlled drug delivery systems, biomaterials must fulfill a number of specifications. They must be inexpensive, easy to process, non-toxic, biocompatible, and biodegradable. The biomaterial's wide range of applications is facilitated by its capacity to create diverse drug delivery structures with varying morphologies, including films, gels, foams, microparticles, and scaffolds [28]**.** Furthermore, the drugs ought to be able, to be released under strict control [29]**.** Silk is a natural polymeric biomaterial that can meet these needs. Because of its special structural characteristics, capacity for self-assembly, mechanical strength, processing flexibility, biodegradability, and biocompatibility [29]**.** A number of tyrosine residues and active amino groups are present in the SF, which can be used to modify the surface for a variety of biomedical applications as well as conjugate drugs, diagnostic agents, and targeting ligands [30]**.** Silk fibroin nanoparticles have been validated as carriers for cytotoxic drugs, such as paclitaxel, and their loading efficiency has been assessed using UHPLC-MSMS [31]**.** Fibroin nanoparticles are useful for delivering a variety of therapeutics because they can be used to encapsulate different drugs and proteins. Research on the use of fibroin nanoparticles in drug delivery systems is still on-going, to maximize their characteristics and investigate potential uses [15]**.** To properly comprehend and utilize fibroin nanoparticles for drug delivery, more study is required.

5. UTILIZATION OF FIBROIN AS NANOPARTICLES

SF Nanofiber possesses many special qualities such as its high biocompatibility, biodegradability, and lack of inflammatory reactions making it a prime candidate as a vehicle or substitute material for biomedical use [32]**.** There are several uses for fibroin as a nanoparticle. SF is appropriate for drug delivery systems because, when coated with liposomes, it forms a compact and distinct lamella structure. SF-chitosan nanoparticles show composition-dependent shape variations. SF-chitosan nanoparticles have a heterogeneous population of spheres, polygons, and small cylinders, while pure SF nanoparticles have cylindrical barrel structures. In the context of drug delivery systems and the creation of nanoparticles for targeted therapy, fibroin can be used as a nanoparticle. SF nanoparticles overcome barriers created by synthetic non-degradable nanoparticles made of silicone, polyethylene glycol, and degradable polylactic acid–polyglycolic acid polymers [33]**.** When cytotoxic medications are delivered via SF nanoparticles, breast cancer cell toxicity is maximized, optimal entrapment is achieved, the therapeutic index is improved, and there is little to no collateral damage to neighboring normal cells [34]**.** Different therapeutic compounds, including big and small molecules, proteins, enzymes, vaccines, and genetic materials, can be encapsulated in FNPs. Because of their versatility, FNPs can be administered in a number of ways, including parenterally, orally, transdermally, ocularly, orthopedically, and respiratory means. FNPs have benefits like chemical modifiability, excellent biomaterial qualities, and the capacity to mitigate

unfavorable side effects brought on by the extensive use of pharmaceutical agents [3]. For bone repair, nanocomposite hydrogels containing glycerophosphate, chitosan, silk fibroin, and Cu-BG NPs were effectively created [35].

6. SYNTHESIS & EXTRACTION TECHNIQUES

The silk fibroin from the cocoon of silkworm *Bombyx mori* is processed and degummed to remove the sericin component, it can be electrospray, dissolved, or gelled to create nanoparticles [34]. There are several ways to create SF nanoparticles, including coacervation, desolvation, and nanoprecipitation methods. By using these techniques, SF is precipitated to create nanoparticles from its solution [36]. It also includes freeze-drying, dialysis, and centrifugation. Through these methods, the nanoparticles can be extracted from the solution and obtained in a dry form [20]. The silk/ionic liquid solution (SIL) can be quickly dissolved in polar organic solvents to produce SF nanoparticles [37]. Acetone was utilized as a desolvating agent in the nanoprecipitation technique to create SF nanoparticles. Acetone, an organic solvent, was utilized to precipitate the SF solution, resulting in the formation of nanometer-sized fine particles [38].

7. ASSAYS TO DETERMINE THE PROPERTIES OF FIBROIN NANOPARTICLES

There are various methods used to determine the properties of fibroin nanoparticles, but only few of them are mentioned here:-

Particle size analysis: It is possible to measure the diameter of SF nanoparticles using methods like scanning electron microscopy or dynamic light scattering [39].

Zeta potential measurement: The surface charge of SF nanoparticles can be determined through zeta potential analysis, which is crucial for their stability and interactions with other molecules. Z potential gives us information about the distribution and balance of surface charges within nanocomposites [40].

Morphology characterization: It is possible to see the morphology and surface structure of SF nanoparticles using scanning electron microscopy [20].

Thermal analysis: Analysis methods like differential scanning calorimetry can be utilised to investigate the stability and thermal behavior of SF nanoparticles [41].

Drug release studies: Drugs or enzymes can be loaded into SF nanoparticles, and techniques like UV-Vis spectroscopy and high-performance liquid chromatography can be used to study the release kinetics of these compounds [42].

Biocompatibility assessment: Tests for cell viability, like MTT or Live/Dead staining, can be used to assess how well SF nanoparticles interact with various cell types [43].

Surface charge determination: Zeta potential analysis is one technique that can be used to measure the surface charge of SF nanoparticles, providing information about their stability and interaction with biological systems [44].

8. STRENGTHS AND LIMITATIONS OF FIBROIN NANOPARTICLES AS A DRUG DELIVERY SYSTEM

FNP-s have a wide range of applications in medicine because of their special chemical, biological, and physical properties. The high degree of biocompatibility of FNP-s with the human organism is one of its most significant features. Significant advancements in tissue engineering, cancer treatment, controlled drug delivery, bone, eye, and skin regeneration have been made possible by the use of FNP-s [45]. Enhancing SF through genetic modification techniques has demonstrated significant potential to confer new attributes upon SFN-s, such as high-level cell targeting and cell-entry efficiency, which may effectively foster the use of SFN-s for drug delivery in the field of nano-medicine [46]. It can also be processed into a variety of forms, such as films, hydrogels, sponges, wafers, gauze, particles, and fibers, making it possible for many additional uses [47]. Moreover, it plays a crucial role in human health, one of the most serious types of injuries are burns, which result in a significant global death toll. Following a burn injury, the patient shows increased susceptibility to various photogenic bacteria) [48]. Additionally, the authors demonstrated the protective activity against infections in vivo using mice as an animal model, underscoring the viability of using SF-based dressings for accelerated tissue healing [49].

Fibroin has certain drawbacks that must be addressed despite its many benefits as a DDS used in many administrative routes. Fibroin, being a protein, is susceptible to proteolytic attack by immune system components like giant cells and macrophages. This can result in the release of drugs off target because the granuloma forms inside these cells and gets encapsulated [50]. Fibroin, like other natural products, can be extracted from a variety of sources, each batch's properties vary slightly due to differences in the post-translational process between different species and individuals. FNP-s alone are not the intended DDS, despite showing great promise in protecting the encapsulated medications, enhancing their stability, and extending their release profiles. As a result, the unspecific targeting may result in systemic toxicity and low therapeutic efficacy [3]**.**

9. FUTURE OUTLOOK

Fibroin, particularly FNPs, has a strong propensity to be the preferred delivery mechanism for a variety of therapeutic agents, such as genes, vaccines, protein drugs, and small molecule drugs. In addition, a variety of FNP delivery methods, including parenteral, oral, transdermal, ocular, local bone implantation, and respiratory, have been studied. Given the advantageous characteristics of FNP, more research ought to concentrate on the less explored but potentially useful pathways, specifically the respiratory and ocular systems. Lastly, and perhaps most importantly, the majority of research on FNPs is based on in vitro and in vivo experiments; therefore, additional clinical trials ought to be carried out to possibly bring FNP use to the market [16]**.**

10. CONCLUSION

Silk fibroin (SF) is a natural protein polymer possessing various properties that make it compatible with drug delivery systems. Due to these unique features viz; good biocompatibility degradability and non-toxicosity silk fibroin is considered a novel drug delivery system. SF is extracted from silk fiber by a process of degumming which removes sericin there by yielding silk fibroin. Various other methods are also used for the extraction of fibroin additionally which is regenerated into silk fibroin nanoparticle (SFN). SF can be used as a nanoparticle in varied fields. SFN'-s can encapsulate different drugs and proteins and it is useful for delivering a lot of therapeutics. Additionally, it acts as a carrier of cytotoxic drugs. It is possible to determine the diameter, surface charge, morphology & surface structure, stability, and thermal behavior, the release kinetics of drugs, cell viability, interaction with biological systems. In conclusion, SFN may act as an effective carrier to support future drug delivery system applications*.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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