Review

RECENT UPDATES OF SILK BASED NOVEL DRUG DELIVERY SYSTEMS

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ABSTRACT

Silk is natural protein basically comprised of fibroin and sericin.Major resource of silk is Oak tasar silkworm *Antheraea proyeli*, Muga silkworm *Antheraea assamensis,* tasar silkworm *Antheraea proyeli*, mulberry silkworm *Bombyx mori* and Eri silkworm *Philosamia ricini*.Special features like mechanical toughness, self-assembly, biocompatible, biodegradability and processing flexibility or low immunogenicity proteins are offering their utility for gene and drug delivery.Silk Fibroin basednanoparticlesare prepared by techniques like electros praying, salting out, supercritical fluid technology, desolvation and mechanical comminution.Each method have a significance important in preparation of Silk Fibroin based nanoparticles for drug delivery.Silk fibroin have broadly used 3D-silk fibroin scaffolds, microspheres, films, nanoparticles, Nano fibers, biomedical sutures, for coatings,sponges, biomaterials for implants and tubes as well as for drug delivery. Patents for silk based drug delivery applications have attracted the researcher involved in drug delivery area.

KEY-WORDS: Silk Worms,Silk Proteins, preparation methods,

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INTRODUCTION:

Silk being a natural protein fiberwhich controlledgenerally of fibroin andmade by certain insect larvae to form cocoons. These silk protein molecules retain bio-degradable,bio-compatible and self assembling properties which are natively improvedwithgene delivery or offering utility for drug and genetic engineering.Someproperties of silk-based material like solubility, biodegradability, and mechanical properties, can be managed by employing the secondary structure.Innovative engineering techniques together with mild all-aqueous progressions consume expanded range of submission tonucleic acid therapeuticsand complexprotein. Silk attained from silkworm is used in biomedical sutures for decades[1,2]SF matrices were confirmed to positively deliver protein drugs and reserve their effectiveness. Silk fibroin havebeenrecommendedfor deliveron the technique viadrug delivery inside the usage of hydrogels porous 3D scaffolds and films. The best characterization silks are the cocoon silk cultivated from the silkworm dragline silk from the spider Bombyx moriand Nephila clavipes.[3,4]Targeted delivery can be attained Hybrid and merged silk-based materialscomprisingsome biopolymers, which has not been widely studied. It should deliver applicable mechanical, or naturalpossessions for not only drugdelivery and gene delivey.Ithas alsointended forregenerative medicine or medical imaging and tissue engineering. Silk are characterized in three types[5].Silk I. water soluble whichhave composed of combination of a-helix and b-turn structures,andrandom coil, Silk II, categorized by a majority of b-sheet which are stable and water insoluble fibroin; Silk III, considered as a-helix and usually found at the water or airinterface.[6,7]Silk fibroin encompassexceptional potential as a carrier for sustained or controlled release of cargo. Silk has importance used role in human body in the form ofsuture material.

Types of Silk Fibroins

1. Silkworm Silk fibroins

Silk fibroin is used as biomedical sutures from ancient time. It is also been used in textile production for clothing, due to the fact that silkworms are easier to domesticate.[8] The core progression in the heavy chain contain the alanine-glycine repeats. In the silkworm cocoons, two fibroins are sheathed in a glue-like, sericin coat proteins, to form the composite fibers of the cocoon. The mainlypremeditated silkworm silk proteins, contain two major components, heavy chain (~325 kDa) and light (~25 kDa) fibroins. Various methods are now used to extract and regenerate silk fibroin .Several silk based biomaterials, such as silk films ,porous scaffolds electrospun Nanofibers, and hydrogels can be processed from silk solutions.[9]

2. Spider Silk Fibroins

*Nephilaclavipes*is is the most universal and commonly deliberate spider silk in terms of structure .Function is dragline silk which is veiled as a mixture of two proteins from focused columnarepithelial cells of the most important ampullate secretor of weaver spinning spiders. The molecular weights of Silk proteins range from 70 to 700 kDa depending on source. Partial cDNAclones programming the two types of dragline silks had been secluded and analyze from two species of orb-web weaving spiders, *N.clavipes*(MaSpI and MaSpII) *and Araneusdiadematus*(ADF-3 and ADF4).[10]The silks proteins are characterized for block copolymers, tranquil of large hydrophobic blocks with highly

preserved repetitive inorder consisting of small side-chain amino acids,like alanine and glycine, with prevailing short hydrophilic block with extramultipartprogression.Which havebeen preparedvia amino acids with charged amino acids and larger side chain. The hydrophobic blocks form beta-sheets and physically cross-linked crystalline domains in silk fibers. The imposing tensile strength of silk fibers is due to the presence of hydrophobic or less ordered hydrophilic regions.Silk fibers are combination with chain direction achieved during revolving.[11]

TYPES OF SILKWORM

There are five types of silk collected fromaltered species of silkworms:-

1) Mulberry

The large of the commercial silk are formed cutting-edge in world originatesafter the diversity and often silk generallyfamous as mulberry silk. Mulberry silkare obtained from the silkworm, *Bombyxmori*[12,13] These silkworms are entirely cultivated and reared inside. In India, the foremost mulberry silk cultivated states are, West Bengal, Karnataka,Tamil Nadu, Andhra Pradesh Andhra Pradesh,and Jammu& Kashmir.[14]

2) Tasar

Tasar (Tussah) is the copperishcolour, silk mostly used for fittings and interiors. It is fewer shiny than mulberry silk.Tasar silk is generated by the silkworm, *Antheraeamylitta*which mostlyincrease on food plants Asan and Arjun. In India, tasar silk is largelyformed in the states of Chhattisgarh, Orissa, and Jharkhand, besides Maharashtra, Andhra Pradesh and West Bengal. Tasar culture is the key continue for lots of a ethnic community in India.[15]

3) Oak Tasar

It is a greater diversity of tasar generated by the silkworm, *Antheraeaproyeli*. In India which are providedfor natural food trees of oak, initiate in profusion in the sub-Himalayan tie of India covering the states of Himachal Pradesh, Jammu & Kashmir, Assam, Uttar Pradesh,Meghalaya,china and Manipur.China is the most imperativeinventor of oak tasar in the world.Tasarderives from altered silkworm which is known as *Antheraeapernyi*.[15]

4) Eri

It's also well-known as Errandi or Endi, Eri has a multivoltine silk spin from open-ended cocoons, nothing similarfurthervariations of silk. Erisilk is the product of the cultivated silkworm, *Philosamiaricini*that feeds mostly on castor leaves. Eri society is a domestic activity proficient mostly for delicacy for the tribal and protein rich pupae.The silk is worn native for production of *chaddars*(wraps) for have used by these tribals.In India, society is expert mostly in the Assam and north-eastern states .[10,16]

5) Muga

Muga is golden yellow colour silk which are privilege of India and the pleasure state of Assam. It is collected from semi-cultivated multivoltine silk*Antheraeaassamensis*. These silkworms forage on the fragrant leaves of Soaluplantand are raised on trees similar to that of tasar. Muga silk is mostly cultivated in the state of Assam and basic part of the tradition. The muga silk as high value creation is used in products like Machala'sand sarees.[12,17]

RECOMBINANT SILK PROTEINS

In the previous few years ago, many methods had been used in thoughtful silk inheritance, structures and biophysics cloning. Silk proteins personalized via

genetic engineering can also be planned to present original features at the side of native properties.[18]

1. Silkworm Variant

Silkworm silk is obtained from *B. mori*silkworm & elastin obstruct copolymers or silk elastin similar to proteins constructed by recombinant DNA techniques. Silk had been utilized as gene and drug release systems forming hydrogels to liberate adenovirus containing writer genesincrease the cell-adhesive facility of silk-fibroin partial collagen and fibronectinsequences.Silk fibroin from natural silkworm *Anaphe*has a large amount simpler amino acid composition in evaluation to *B. mori*silkworm silk fibroin . *Anaphe*silk fibroin may be a suitable for the design and formulation for the recombinant proteins.[19,20]

2. Spider Variants

A spider silk sequence was customized to have methionines adjacent to the polyalanine (beta sheet forming domain) sequence and derived from the silaffin protein of the diatom *Cylindrothecafusiformis*.Silk based amphiphilic have been developed to improve the transfection efficiency through integrin-mediated endocytosis .The designs can be extensive to further control targeting,stability and size or related some needs for gene delivery. [21]

Table No 1. Comparison of silk proteins from the cocoons of mulberry silkworm *B.mori*

Protein	Fibroin	Sericin
Composition	Amino acids in a heavy chain and a light chain in a 1:1 ratio linked by disulfide bonds.[22]	Polypeptide polymer consisting of18 amino acids [23]
Type of protein	Structural protein	Glue-like protein
Proportion of cocoon	70%	20-30%
Structural Properties	Hydrophobic; β-sheets and α-helices	Hydrophilic; remains in a partially unfolded state, with high proportion of random coil structure [23]
Molecular weight	26-370 kDa	24-250 kDa
Major properties	Biocompatible, Biodegradable,Crystallinity, Mechanical Properties, Opportunity for Chemical Functionalization [24]	Antibacterial, UV-absorbing, high Moisture Absorbancy, Antioxidant, Antitumor, Wound-healing[25]
Novel applications	Drug delivery, Tissue engineering, Implant coating, imaging and diagnostics[24]	Food, Cosmetics, Drug delivery, Medical and Pharmaceutical Industries
Advantages for use in drug delivery	Aqueous processing, controllable biodegradation	Capable of carrying both hydrophilic and hydrophobic drugs

PROPERTIES OF SILK FIBRES.[26,27]:

Table No 2. Physical & Chemical Properties of Silk Fibers.

Physical properties	Chemical Properties :
Silkworm includes a triangular cross section shape with curved corners, 5-10 µm wide. The Fibroin made up of generally beta-sheets, due to presence a 59 amino acid repeat sequence with several variations.	Silk is opposed to mainy mineral acids, apart from for sulfuric acid, in which dissolve it.
Silk have numerous synthetic fibers, unlike soft texture that is not slippery, and smooth.	Silkworm consists of mainly two main proteins fibroin and sericin.
Silk has susceptible to static cling and poor conductor of electricity.	The high percentages (50%) of glycine, which are few amino acid, allow stiff stuffing. Fibers are opposed to breaking and tough.
It have also used in favor of the denier and measurement of linear density in fibers	Fibroin consist of the heavy amino acids chains Gly-Ser-Gly-Ala-Gly-Ala and developed beta sheets.
Silk has virtually Thermally stable between 140°C to 175° C	

Chemical Structure of Silk Fibroin :

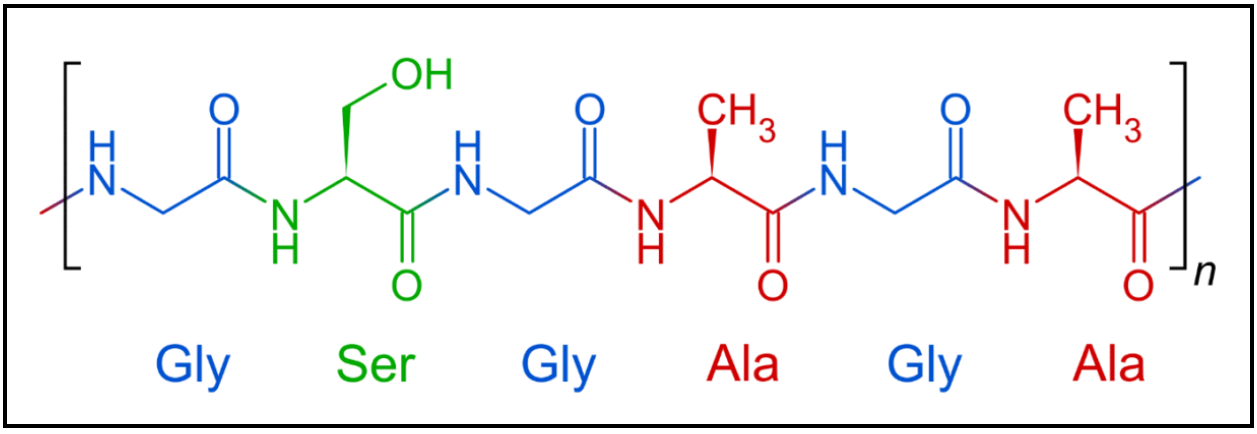

Figure 1.Chemical Structure of Silk Fibroin.[28]

Procedure for Extraction of Fibroin and Sericin.

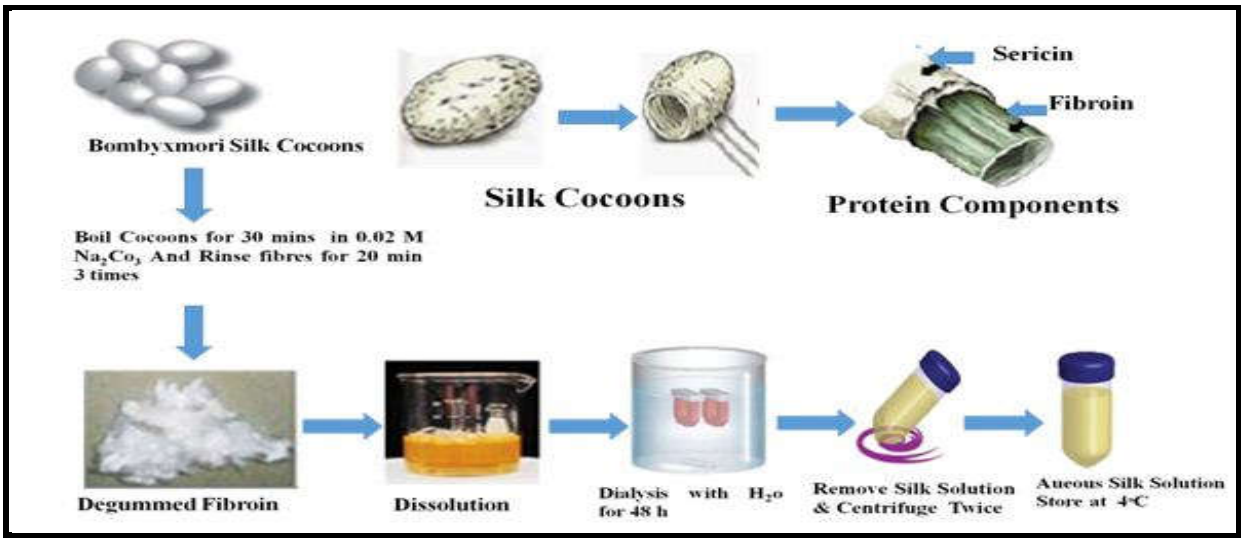

Figure 2. Extraction of fibroin and sericin from the cocoons of *B.mori*silkworms.[24,29]

Technique of Preparation for Silk Fibroin Based Nanparticles.[30]

Numerous methods are available for the formulation of SF-based nanoparticles or microspheres, like salting out, desolvation, electros prayingand mechanical comminution,or PVA blend technique. The high protein nature and molecular weight of Silk Fibroin make the preparation of Nano formulation difficult to control. Furthermore Silk Fibroin tends to self-assemble into gels or fibers upon exposure to high shear,pHchange,salt and heat.

Table 3. Methods for Preparation of silk fibroin-based Nanoparticles.

Sr. No.	PreparationMethods	Advantages	Disadvantages	Particle Size
1.	Salting out	Low cost; high yield;Avoids use of toxic solvents and safe operations	Salting out agents residue.	486~1200 nm
2.	PVA blend film method	Time and energy efficient. Mild operation conditions or safe to manipulate.	PVA residue.	500 nm~2 mm
3.	Desolvation	Small particle size;	Organic solvent residue	35~125 nm

		Simplicity of operation.		150~170 nm
4.	Electric fields	Mild operation conditions;	Particle with big size.	200 nm~3 µm
5.	Electrospraying	Controllable particle size; Simplicity of operation.	To induce insolubility of SF	59~75 nm
6.	Mechanical Comminution	Easy to scale up	The impurities and any grinding aids need be removed	~200 nm

Salting out

Salting out Method involves thesalting out of protein solution to obtain protein co-acerbates. Proteins has hydrophobic and hydrophilic parts. Hydrophobic parts could be interrelated through the water molecules and permit the proteins to attained hydrogen bonds among the adjoining water molecules. By the increasing of the salt concentration the ions of salt attract a few of water molecules and as a resulting in the confiscation of the water fence among protein molecules.The protein molecules amassed together by formed hydrophobic exchanges with each other and the solution. Formation of Silk Fibroin nanoparticles with normal diameter of 486~1200 nm in aqueous development through salting out method. [31,32]

Desolvation

Generally thistechnique is used to prepare protein-based nanoparticles. The desolvation or simple coacervation process decrease the solubility of the protein foremost to phase separation. The affixing of desolvating agent leads to conformation modify in protein structure ensuing in precipitation or co-acervation of the protein. Throughout the phase separation and phase by means of a colloidal constituent or co-acervateand subsequent stage via solvent or non-solvent mixture are formed. A steady particle size is reached after an original development stage that extra desolvation exclusively lead to enlarged element yield. [33,34,35]

Mechanical Comminution

Comminution is the decrease of solid resources as of single usual particle size to a lesser average crushing,particle size, or grinding and milling *etc.* The method usually involves high energy wet or dry milling through the adding up of mill aids and naturally use milling period from several hours up to lots of days.The process is simple to function and scale up. Technique still experience from difficulty in ensure to all the particle be crushed properly. Extended mill time as well result in extra milling impurities. Furthermore, the constituent part size division is wide.[36,37]

PVA Blend Film Method

Silk Fibroin constituent part with convenient particle size (500 nm~2 mm) as well as shape via PVA as a continuous phase to separate Silk Fibroin solution into microspheres or nanoparticles in Silk fibroin: PVA blend films at a weight ratio from 1/1 to 1/4. The procedure was based on phase separation between SF and polyvinyl alcohol (PVA).The SF/PVA blend solution have dried out into a film firstly. After that water insoluble Silk Fibroin particle

might be fabricated via film dissolution in water and subsequent centrifugation toward take away PVA. The process was easily because the process in used with PVA and water an FDA-approved substance. Through regulate the concentration of SF and PVA or take up ultra-sonication on the blend solution. The SF particles with diverse particle size can be prepared. Drug could be loaded into Silk Fibroin particle via addition model drugs in the original Silk Fibroin solution. These Silk Fibroin element contain probable as drug carriers in field of biomedical applications.[38]

Electrospraying

Electrospraying is a technique of liquid atomization via means of electrical *forces* with is an emerging process for the fast and elevated during put making of nanoparticles. In electros praying process, the liquid smooth out of a capillary nozzle, preserved at high electric potential and forced with the electric field to dispersed into small droplets.[39]

Electric Fields

In this process the arrangement of the Silk Fibroin nanoparticles through size of tens of nanometers is significant pace. Underneath electric pasture the nanoparticles comprehensive to form microspheres or nanoparticle on top of positive electrodes put off inter molecular self-assembly of Silk Fibroin in impartial solution.[40]

METHODS OF PREPARATION FOR SILK FIBROIN-BASED MICROSPHERES.

The following method is used for preparation of silk micro-spheres which can be used to encapsulate and release growth factors with small molecules and therapeutic compounds.

1. **Prepared byDOPC Method[41]**

- ✓ Aqueous silk solution, 8% (wt/vol)
- ✓ 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC
- ✓ Chloroform
- ✓ Methanol ,Nitrogen gas
- ✓ Drug, small molecule or protein of interest
- ✓ Ultrapure water

2. **Prepared by PVA Method[42,43]**

- ✓ Aqueous silk solution, 8% (wt/vol)
- ✓ PVA Polyvinyl Alcohol (MW 30–70 kDa;
- ✓ Ultrapure water

APPLICATIONS OF SILKWORM[44]

Table No 4. Formulations and applications of Silkworm

Sr.No	Application	Tissue type	Formulation
1.	Drug delivery	1. Drug Delivery 2. Small Molecules 3. Growth Factor delivery	1. Spheres 2. Mcro- or nanoparticle 3. Spheres
2.	Implant devices	1. Anterior cruciate ligament 2. Mandibular defects	1. Fibers 2. Aqueous sponges
3.	Disease models	1. Breast cancer	1. HFIP sponges

4	Tissue engineering	1. Tissue engineering 2. Corneal 3. Cervical tissue 4. Cartilage 5. Skin 6. Vascular tissue	1. HFIP sponges or Hydrogels 2. Patterned silk films 3. Aqueous sponges 4. HFIP sponges or Hydrogels 5. Electrospun fibers 6. Tubes or Electrospun fibers

APPLICATION

Preparation of silkworm silk- based biomaterials

Silk has been used in biomedical sutures since its mechanical strength and biocompatibility. Though silk associated with pollutant sericin proteins is a probable allergen reason. The degradation invention of silk fibroin proteins among beta-sheet structures from the action of proteases like alpha-chymotrypsin have recently be details or no cytotoxicity was experiential to neuron cells *in-vitro*.[45,46]

Films

Silk films have been used with different covalent decoration of functional peptides like implants for drug Delivery and bone formation. For parathyroid hormone (PTH), bone regeneration, RGD, and BMP-2, can be directly immobilized on silk films by carbodiimide chemistry. Differentiation of human bone marrow-derived stem cells cultured with the bedecked silk films can be induced by immobilized BMP-2. Moreover, the usefulness of silk films to support long-term adenosine release from adenosine kinase deficient embryonic stem cells has been examined. Silk films adorned with bioactive molecules can be used for local drug delivery through direct implantation.[47,48]

Nanoparticles

Silk-based nanoparticles have been prepared in nature derived silk fibroin support nanoparticles less than 100 nm) for local and sustained therapeutic curcumin delivery toward cancer cells.Curcumin loadedsilk nanoparticles prepared in various proportions with pure silk fibroin or silk fibroin with chitosan and PVA were spherical, stable, negatively charged, 150-170 nm in average diameter as well as showed no toxicity. The silk-based nanoparticles enclose curcumin demonstrate a higher effectiveness against breast cancer cells also contain potential to care for *in-vivo* breast tumors via local or sustained and long term therapeutic delivery.[49,50]

Coatings

Coatings of silk fibroin contain studied to give interface for biomaterials. The forceful force of self-assembly to form coatings is electrostatic and some hydrophobicInteractions. The elasticity of silk based coating have been investigated using an aqueous step wise evidence process with *B. mori*.The width of one layer was reported to be around 10 nm while put down from a one mg/mL aqueous solution of silk. The secondary arrangement of silk fibroin in the coating was regulated to manage the biodegradation rate, which indicates layer thickness and numbers of

layers and secondary structure of the layers.[51]Nano layer coatings of silk fibroin is used to enclose model compounds of small molecule drugs or therapeutically applicable proteins. Multilayered silk based coatings have been urbanized or used as drug carriers and delivery systems to estimate vascular cell comeback to heparin and clopidogrel.[52, 53]

Microspheres

Silk fibroin microspheres had been prepared via different techniquelike spray-drying, desolvation, electric field though, the size of the microspheres had above 100 µm, which is suboptimal intended for drug delivery. Further technique are prepare silk microspheres include lipid vesicles as templates to professionally load bioactive molecules used for local controlled releases be reported recently. The lipid is then removed by methanol or NaCl, resulting in silk microspheres consistsof beta-sheet structure and around 2 µm in diameter .[54,55]

Implants, tubes and scaffolds

Silk-based Three D scaffold loaded through one morphogenetic protein-2 (BMP-2) to encourage human bone marrow stromal cells to undertake estrogenic differentiation due to their biocompatibility and mechanical properties. Horseradish peroxidase (HRP) enzyme gradients beimmobilized on silk 3D scaffolds to arrange original functional scaffolds as well as regional patterning of the rise to manage cell and tissue outcomes currently studies show adenosine release through silk based implants to the brain have been studied for refractory epilepsy treatments.[56.57] Silk based implants releases adenosine demonstrated therapeutic ability or together with the controlled release of adenosine over a period of two weeks through slow degradation of silk or biocompatibility and the release of prearranged dose of adenosine . Nerve growth factor (NGF) loaded silk fibroin nerve encompass been studied to guide the developing of axons and physically defend the axonal cone for peripheral nerve repair .Silk fibroin scaffolds have insulin-like growth factor I (IGF-I) have been formulated for controlled IGF-I release in the situation of cartilage repair [58]

Tropical tasar silkworm *Antheraea mylitta* had also estimated for *in-vitro* drug release along with for the study of cell surface interactions.Silk based micro molded matrix hold up a significant development in cell attachment or spreading mitochondrial activity along with proliferation through feline fibroblasts in association to polystyrene plates as controls.[59]

Table No 5. Silk Fibroin Patents

Sr No.	Publication No	Inventors	Title	Reference
1.	EP2403551 A2	David L. Kaplan, Bruce Panilaitis, Eleanor M. Pritchard,FiorenzoOmenetto, Jordan Axelrad	Silk fibroin systems for antibiotic delivery	60
2.	WO 2013159101 A1	Evangelia BELLAS, Amanda BARYSHYAN, Lindsay WRAY, David L.	Silk fibroin based care compositions	61

		Kaplan		
3.	US 20150164117	Kaplan, David L. (Concord, MA, US) Omenetto,Fiorenzo (Lexington, MA, US)	Encapsulation of fragrance and/or flavors in silk fibroin biomaterials	62
4.	US5252285 A	Robert L. Lock	Process for making silk fibroin fibers	63
5.	US8178656 B2	David L. Kaplan, Meinel Lorenz	Silkbased drug delivery system	64
6.	WO2013142119 A1	David L. Kaplan, Tuna Yucel, Michael L. Lovett, Xiaoqin Wang	Silk reservoirs for drug delivery	65

Table No. 6 Silk fibroin based Marketed Formulation:

Silk fibroin based formulation are marketed in mainly in China in Powder form.

Sl. no	Formulation	Brand Name	Type	Function	Business Type
1.	Powder	Silk Peptide Powder	Watersoluble polypeptide substance	Skin wound healing.	HuzhouXintiansi Biotech Co., Ltd. China
2.	Liquid	Silk Peptide Liquid	Water soluble polypeptide substance	Hair Care Function	China tiansi biotech co.,ltd, seoul korea
3.	Powder	Silk fibrin powder	macromolecular silk fibrin	natural moisturizing, absorb part of ultraviolet ray	HuzhouXintiansi Biotech Co., Ltd. China
4.	Powder	Silk fibroin powder	Solvent Extraction	nourishing skin	Xi'an Lyphar Biotech Co., Ltd, China.
5.	Silk fibroin powder	ZhongYun	Solvent Extraction	natural and promoting healing of skin wounds	Trading Company Shaanxi, China
6.	Amino acids silk protein	Lingeba	Silk Fibroin extract	Smoothness for natural hair	Hangzhou, zhejiang,China

CONCLUSION

Silk Fibroin is an attractive polymer for drug delivery of bioactive compounds for the sustained or controlled drug delivery. Silk Fibroin based matrixes , micro particles, nanoparticles were prepared from aqueous solutions were stable, negatively charged, spherical and showed no toxicity of the fibroin protein polymer and prepared by the different techniques. Crystalline can be controlled and induced by action with different solvents. Drug release from these coating have sustained or controlled by number of layers, layer thickness and secondary arrangement of the silk fibroin layer.

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