Applications of Silk in Drug Delivery: Advancement in Pharmaceutical Dosage Forms

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ABSTRACT: Silk is one of the important natural protein fibre produced by certain insect larvae, Major source are mulberry silkworm *Bombyx mori*, Tasar silkworm *Antheraea mylitta*, Oak tasar silkworm *Antheraea proyeli*, Eri silkworm *Philosamia ricini* and Muga silkworm *Antheraea assamensis*. Silk is having pleotropic effect of being novel aid in drug delivery because of its properties like self-assembly, mechanical toughness, processing flexibility, biodegradability and biocompatibility. Silkworm silk has been widely used as biomedical sutures, 3D- silk fibroin scaffolds, films, nanofibers, nanoparticles, microspheres, for coatings, microneedles etc. Present review article covers these with sufficient information. © 2011 IGJPS. All rights reserved.

KEYWORDS: NDDS; Silk Proteins; Silk Worms.

INTRODUCTION

Silk is a natural protein fibre which is composed mainly of fibroin and produced by certain insect larvae to form cocoons[1].These silk protein molecules posses biodegradable, biocompatible, self assembling properties that are genetically modified using genetic engineering, offering utility for drug and gene delivery and properties of silk-based material like mechanical properties, solubility, and biodegradability, can be controlled by manipulating the secondary structure.[2] Silk obtained from silkworm is used in biomedical sutures for decades and has recently achieved Food and Drug Administration approval for expanded biomaterials device utility. With the diversity and control of size, structure and chemistry, modified or recombinant silk proteins can be designed and utilized in various biomedical application, such as for the delivery of bioactive molecules[3].Silk proteins are obtained from variety of insects and spiders, which forms fibrous material in nature such as silkworm cocoons and spider orb webs.[4] Spider silk has outstanding mechanical & structural properties, despite its light and delicate appearance, like high strength and exceptional toughness. Silk is twice as stretching nylon and eight times more strong than steel.[5]The basic function of these silk proteins are :-
a. Development (Cocoons)
b. Capturing prey (Spider webs)
c. Safety lines(Spider draglines)[6]

TYPES OF SILK FIBROINS
1)   Silkworm Silk fibroins
Silkworm fibroin is used as a biomedical sutures from many years, it also used in textile production for clothing, because of the fact that silkworms are easier to domesticate and obtain silk in comparison to spider silks. The silk fibroin from the cocoon of silkworm *Bombyx mori*, the most studied silkworm silk proteins, contain two major components, light (~25 kDa) and heavy chain (~325 kDa) fibroins. The core sequence in the heavy chain include alanine-glycine repeats. In silkworm cocoons, these two fibroins are encased in a sericin coat, glue-like proteins, to form the composite fibers of the cocoon. Various methods are used now a days to extract and regenerate silk fibroin [7][8],and several silk-based biomaterials, such as silk porous scaffolds, silk films, hydrogels, and electrospun nanofibers, can be processed from silk solutions.

2)   Spider Silk Fibroins
Nephila clavipes is the most common and widely studied spider silk in terms of structure and function is dragline silk which is secreted as a mixture of two proteins from specialized columnar epithelial cells of the major ampullate gland of orb-weaver

spinning spiders [5][9]. The molecular weights of these proteins ranges from 70 to 700 kDa depending on source. Partial cDNA clones encoding the two types of dragline silks have been isolated and analyzed from two species of orb-web weaving spiders, *Araneus diadematus* (ADF-3 and ADF4) and *N. clavipes* (MaSpI and MaSpII) [10][11]. These silk proteins are characterized as block copolymers, composed of large hydrophobic blocks with highly conserved repetitive sequences consisting of short side-chain amino acids, such as glycine and alanine, with intervening small hydrophilic blocks with more complex sequences that consist amino acids with bulkier side-chain and charged amino acids [12]. The hydrophobic blocks form beta-sheets, or physically cross-linked crystalline domains in silk fibers. The impressive tensile strength of silk fibers is due to the ordered hydrophobic and less ordered hydrophilic regions, in combination with chain orientation achieved during spinning [13].

RECOMBINANT SILK PROTEINS
In the last few years, various methods have been used in understanding silk genetics, structures and biophysics [10][14]. Cloning, modification and expression of native and synthetic silks has been achieved in a variety of host systems using synthetic oligonucleotide [11]. Silk proteins modified by genetic engineering can also be designed to display new features alongside native properties

1) Silkworm Variant
Silkworm silk which is obtained from *B. mori* silkworm and elastin block copolymers, silk-elastin-like proteins constructed by recombinant DNA techniques, which have been utilized as gene and drug delivery systems, by forming hydrogels to release adenovirus containing reporter genes [15]. To increase cell-adhesive ability of silk fibroin partial collagen and fibronectin sequences were inserted into silk fibroin from *B. mori* silkworm [16]. The recombinant silk proteins were produced by transgenic *B. mori* silkworm [16]. Silk fibroin from wild silkworm *Anaphe* has a much simpler amino acid composition in comparison to *B. mori* silkworm silk fibroin . *Anaphe* silk fibroin may be a suitable candidate to design silkworm silk fibroin-mimetic recombinant proteins. Fusion proteins of silk fibroin from *Anaphe* and cell-binding motifs have been designed and synthesized using *E. coli*, to generate biomaterials with high cell adhesive ability when compared with collagen and *Anaphe* silk protein.[17]

2) Spider Variants
To control self-assembly of beta-sheet structures in silk, a spider silk sequence was modified to contain methionines adjacent to the polyalanine (beta sheet forming domain) sequence [18]. The R5 peptide which is being derived from the silaffin protein of the diatom *Cylindrotheca fusiformis*, which forms reproducible nanostructures and silica precipitation from silicic acid using species specific peptides known as silaffins, was added into the silk to generate nanocomposites for bone regeneration . For new gene delivery systems, silk-based amphiphilic block copolymers with poly(l-lysine) have been developed to enhance the transfection efficiency via integrin-mediated endocytosis [19][20]. These designs can be extended to further control targeting, size, stability and related needs for gene delivery. The recombinant silk-like polymers mentioned herein have demonstrated utility as advanced

highly tailored or designed biomaterials with different features – from composite material systems to gene delivery.

TYPES OF SILKWORM
There are five major types of silk obtained from different species of silkworms:-

1) Mulberry
The bulk of the commercial silk produced in the world comes from this variety and often silk generally refers to mulberry silk. Mulberry silk comes from the silkworm, *Bombyx mori* L. which solely feeds on the leaves of mulberry plant. These silkworms are completely domesticated and reared indoors. In India, the major mulberry silk producing states are Karnataka, Andhra Pradesh, West Bengal, Tamil Nadu and Jammu & Kashmir which together accounts for 92 % of country's total mulberry raw silk production[21][22]

2) Tasar
Tasar (Tussah) is copperish colour, coarse silk mainly used for furnishings and interiors. It is less lustrous than mulberry silk, but has its own feel and appeal. Tasar silk is generated by the silkworm, *Antheraea mylitta* which mainly thrive on the food plants Asan and Arjun. The rearings are conducted in nature on the trees in the open. In India, tasar silk is mainly produced in the states of Jharkhand, Chattisgarh and Orissa, besides Maharashtra, West Bengal and Andhra Pradesh. Tasar culture is the main stay for many a tribal community in India[21]

3) Oak Tasar
It is a finer variety of tasar generated by the silkworm, *Antheraea proyeli* J. in India which feed on natural food plants of oak, found in abundance in the sub-Himalayan belt of India covering the states of Manipur, Himachal Pradesh, Uttar Pradesh, Assam, Meghalaya and Jammu & Kashmir. China is the major producer of oak tasar in the world and this comes from another silkworm which is known as *Antheraea pernyi*.[21]

4) Eri
Also known as Endi or Errandi, Eri is a multivoltine silk spun from open-ended cocoons, unlike other varieties of silk. Eri silk is the product of the domesticated silkworm, *Philosamia ricini* that feeds mainly on castor leaves. Ericulture is a household activity practiced mainly for protein rich pupae, a delicacy for the tribal. Resultantly, the eri cocoons are open-mouthed and are spun. The silk is used indigenously for preparation of *chaddars* (wraps) for own use by these tribals. In India, this culture is practiced mainly in the north-eastern states and Assam. It is also found in Bihar, West Bengal and Orissa. [21]

5) Muga
This golden yellow colour silk is prerogative of India and the pride of Assam state. It is obtained from semi-domesticated multivoltine 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. Muga culture is specific to the state of Assam and an integral part of the tradition and culture of that state. The muga silk, an high value product is used in products like sarees, mekhalas, chaddars, etc. [21]

PROPERTIES OF SILK FIBRES

Physical properties

- Silk fibers from the *Bombyx mori* silkworm have a triangular cross section with rounded corners, 5-10 μm wide. The fibroin-heavy chain is composed mostly of beta-sheets, due to a 59-mer amino acid repeat sequence with some variations. The flat surfaces of the fibrils reflect light at many angles, giving silk a natural shine. The cross-section from other silkworms can vary in shape and diameter: crescent-like for *Anaphe* and elongated wedge for *tussah*. Silkworm fibers are naturally extruded from two silkworm glands as a pair of primary filaments (brin), which are stuck together, with sericin proteins that act like glue, to form a bave. Bave diameters for tussah silk can reach 65 μm. See cited reference for cross-sectional SEM photographs.[23]

- Silk has a smooth, soft texture that is not slippery, unlike many synthetic fibers.

- Silk is one of the strongest natural fibers but loses up to 20% of its strength when wet. It has a good moisture regain of 11%. Its elasticity is moderate to poor: if elongated even a small amount, it remains stretched. It can be weakened if exposed to too much sunlight. It may also be attacked by insects, especially if left dirty.

- Silk is a poor conductor of electricity and thus susceptible to static cling.

- Unwashed silk chiffon may shrink up to 8% due to a relaxation of the fiber macrostructure, so silk should either be washed prior to garment construction, or dry cleaned. Dry cleaning may still shrink the chiffon up to 4%. Occasionally, this shrinkage can be reversed by a gentle steaming with a press cloth. There is almost no gradual shrinkage nor shrinkage due to molecular-level deformation.

- Natural and synthetic silk is known to manifest piezoelectric properties in proteins, probably due to its molecular structure.[23]

- Silkworm silk was used as the standard for the denier, a measurement of linear density in fibers. Silkworm silk therefore has a linear density of approximately 1 den, or 1.1 dtex.

Chemical Properties

- Silk emitted by the silkworm consists of two main proteins, sericin and fibroin, fibroin being the structural center of the silk, and serecin being the sticky material surrounding it. Fibroin is made up of the amino acids Gly-Ser-Gly-Ala-Gly-Ala and forms beta pleated sheets. Hydrogen bonds form between chains, and side chains form above and below the plane of the hydrogen bond network.

- The high proportion (50%) of glycine, which is a small amino acid, allows tight packing and the fibers are strong and resistant to breaking. The tensile strength is due to the many interceded hydrogen bonds, and when stretched the force is applied to these numerous bonds and they do not break.

- Silk is resistant to most mineral acids, except for sulfuric acid, which dissolves it. It is yellowed by perspiration.

ADVANTAGES OF SILK PROTEINS AS BIOMATERIALS FOR DRUG DELIVERY

Delivery of bioactive molecules to site of action and drugs providing slow, sustained, controlled release profile is required for many applications. Other than this such delivery system would be more useful if the product were biodegradable, biocompatible, and mechanically durable in nature and could be prepared and processed under ambient aqueous conditions to prevent loss of bioactivity of the drugs to be delivered. Silks can be helpful in addressing these needs, due to the some of the properties like self-assembly, mechanical toughness, processing flexibility, biodegradability and biocompatibility. Various studies has shown that silk is biocompatible and less inflammatory than other common biodegradable polymers such as poly(lactide) and collagen [19]. Another important characterstics of silks is their processability into different material format, such as films, hydrogels, nanofibers, nanoparticles.[20]. The ability to regulate the structure and morphology of silk proteins in an all-aqueous process render this family of structural proteins important candidates for drug delivery applications. The degradation rate can be adjusted by controlling the crystalline state (beta-sheet content) during processing, to regulate the release profile of bioactive molecules. In case of recombinant silk proteins containing the ligand molecules providing the selective delivery to target cells have been generated. In case of cancer treatments drug delivery, silk proteins containing tumor-homing peptides can be designed for specialized delivery using nanoparticles or polyplexes targeting tumor cells. Other target delivery systems without ligands or tumor-homing peptides for direct introduction to tumor cells via injectable hydrogels or implant materials to release drugs or genes can also be considered.

BIOSYNTHESIS OF RECOMBINANT SILK- LIKE POLYMER

The synthesis of recombinant silk-like polymers can be broadly defined in two major steps:

A) Design, construction, and cloning of the genes, and

B) Expression and purification of the protein polymers

A) Construction of silk-like polymer genes

Synthetic genes encoding dragline silk from *N. clavipes* have been successfully constructed, cloned, and expressed. The synthesis of these recombinant genes is based on the repetitive sequences found in native dragline silk genes . The methods for construction of these repeats were previously reported, using smaller oligonucleotide repeats .Thus, the size and sequence of the protein generated can be controlled by the primary sequence synthesized as oligonucleotides (the building blocks). The use of synthetic gene technology to control silk protein size allows for the study on relationships between sequence length and structure-function, along with the study of novel compositions [15]. Partial cDNA sequences from *N. clavipes* encoding the proteins which are repetitive in

nature, such as dragline protein MaSp1 sequence have been cloned and used to construct synthetic genes. Plasmid vectors such as pPT358, pPT317 and pET30a have been used to place the synthetic genes under the control of either bacteriophage T5 or T7 promoters and also to add [His]$_6$ at the N-terminus of the recombinant protein to simplify purification by metal affinity chromatography [24]. Based on these recombinant DNA techniques, many block copolymers containing silk-like sequences have been synthesized by these variants include modified spider silks bioengineered to include RGD cell-binding domains to enhance cell adhesion, inclusion of molecular triggers to control of self-assembly, chimeric silk proteins for controlled mineralization, and silk block copolymers[19]

B) Expression and purification of silk-like polymers

Expression of the encoded proteins in *E. coli* has been successful. *E. coli* have been used as host systems, and yields of proteins were inversely correlated with the size of the synthetic genes. After protein expression, cells are harvested by centrifugation and the cell pellets are resuspended and lysed in denaturing buffer such as urea, guanidinium chloride, or guanidinium thiocyanate [19]. Purification of the expressed proteins has been performed by metal affinity chromatography, such as with nickel-nitrilotriacetic acid After washing the column with denaturing buffer at pH 6.3, the proteins are eluted with denaturing buffer at pH 4.5 (without imidazole). Purified samples are extensively dialyzed against acetic buffer or NH_4HCO_3 solution, and then Milli-Q water [2].

APPLICATION OF RECOMBINANT SPIDER SILKS TO DRUG DELIVERY
Reconstituted spider silk proteins
The reconstituted dragline silk proteins from the spider *Araneus diadematus* have been used to prepare microcapsules for drug delivery using self-assembly of the proteins at an emulsion interface . These microcapsules were suggested to be useful to encapsulate small active ingredients, provided that the active ingredient does not impede the adsorption of the silk and/or that the encapsulation process does not alter the ingredient . Microspheres of bioengineered spider silks, which were derived from ADF4 from *A. diadematus*, were formed by several methods such as dialysis and micromixing [25]. As a result of their material strength, biocompatibility, and the possibility of functionalization via recombinant protein techniques, spider silk microspheres may offer potential for the development of targeted drug delivery systems.

Spider silk-polycation block copolymers
In previous studies, a silk-based block copolymer was generated by combining spider silk consensus repeats with poly(l-lysine) for gene delivery . Poly(l-lysine) is a cationic polymer that interacts with DNA through electrostatic interactions to assemble into polyelectrolyte complexes, is degraded in cells and has been used as an alternative to recombinant viruses for the delivery of pDNA into cells [26]. The silk-based block copolymers formed ion complexes with pDNA, and the sizes were controllable based on the polymer/DNA ratio or molecular weight of poly(l-lysine)

bioengineered into the designs [20]. The sizes of pDNA complexes prepared at copolymer/pDNA ratio of 10 ranged from 310 to 590 nm. Also, the pDNA complexes of silk-based block copolymers with less than 30 lysines showed no cytotoxicity toward human embryonic kidney (HEK) cells. This study demonstrated the feasibility of bioengineering highly designed silk-based pDNA complexes for gene delivery systems; however, the transfection efficiency was too low to be useful for gene vectors.

Spider Silk Polycation- functional peptide multiblock copolymers
Silk-based block copolymers are potentially useful candidates for nonviral gene vector, because various functional peptides such as cell binding motifs (RGD), cell penetrating peptides , signal peptides of virus , and/or tumor-homing peptides can be added as ligands through recombinant DNA techniques, an important advantage of recombinant silk proteins over liposomes and synthetic polymers as gene delivery systems as shown in. Silk-based block copolymers can contain several modules based on the molecular design for specific target delivery, and also regulate sizes, drug release rates, and zeta potentials of complexes of the copolymer and bioactive molecules. Several functional peptides are briefly reviewed, since they may be potentially useful for target gene/drug delivery systems[27][28]

APPLICATIONS OF SILKWORM SILK TO DRUG DELIVERY
Preparation of silkworm silk- based biomaterials
Silkworm silk has been used as biomedical sutures because of its biocompatibility and mechanical strength. However, virgin silk with the associated contaminant sericin proteins is a potential allergen causing a Type I allergic, due to upregulated IgEs in response to the sericins . Once the sericins are properly removed, there is minimal response from the core fibroin structural proteins, as described earlier. The degradation product of silk fibroin proteins with beta-sheet structures from the action of proteases, such as alpha-chymotrypsin, has recently been reported, and no cytotoxicity was observed to neuron cells *in-vitro* [29]. Removal of sericin from virgin silkworm silk is therefore necessary to prepare non-allergic response and non-cytotoxic silk-based materials. Methods to extract and regenerate silk fibroin have been developed cocoons of *B. mori* silkworm silk are boiled for 30-60 min in an aqueous solution of 0.02 M Na_2CO_3 and then rinsed with MilliQ water to extract the sericin proteins. The extracted silk is then dissolved into 9.3M LiBr solution at 60°C, yielding a 20 wt% silk solution. The silk solution is dialyzed in MilliQ water using dialysis membrane with MWCO of less than 3,500 for more than 48 h. According to this basic method, approximately 7-8 wt% silk solutions are obtained. Also, silk sponges, which are obtained from silk solution after lyophilization, can be dissolved in hexafluoro-2-propanol (HFIP). Several silk-based biomaterials can be processed from silk solutions . For instance, aqueous-derived and HFIP-derived silk porous scaffolds have been prepared using salt leaching, gas forming, or freeze drying method. Silk films are prepared by cast or layer-by-layer deposition of silk aqueous

or HFIP solution with various concentrations . Hydrogels of silk fibroin are formed via sol-gel transitions by sonication, vortexing, or the presence of acid and/or ions . Nanofibers of silk fibroin can be prepared by electrospinning . For silk-based materials, methanol can be used to induce beta-sheet structure in the materials, which makes them water-insoluble and slower-biodegradable . Alternatively, water annealing has also been developed for such transitions, avoiding the use of any organic solvents.[30][31]

Implants, tubes and scaffolds

Silk-based 3D scaffolds are attractive biomaterials for bone tissue regeneration because of their biocompatibility and mechanical properties . The 3D silk fibroin scaffolds loaded with bone morphogenetic protein-2 (BMP-2) were successfully developed for sustained release of BMP-2 in order to induce human bone marrow stromal cells to undergo osteogenic differentiation when the seeded scaffolds were cultured *in-vitro* and *in-vivo* with osteogenic stimulants for 4 weeks . Horseradish peroxidase (HRP) enzyme gradients were also immobilized on silk 3D scaffolds to prepare new functional scaffolds including regional patterning of the gradients to control cell and tissue outcomes. Recently, adenosine release via silk-based implants to the brain has been studied for refractory epilepsy treatments . Silk-based implants to release adenosine demonstrated therapeutic ability, including the sustained release of adenosine over a period of two weeks via slow degradation of silk, biocompatibility, and the delivery of predetermined dose of adenosine . Nerve growth factor (NGF)-loaded silk fibroin nerve conduits have been studied to guide the sprouting of axons and to physically protect the axonal cone for peripheral nerve repair . NGF release from the differently prepared silk fibroin-nerve conduits was prolonged over 3 weeks, while the total amount of NGF released depended on the drying procedures used in the preparation of the nerve conduits, such as air drying or freeze drying [32][33][34]. Silk fibroin scaffolds containing insulin-like growth factor I (IGF-I) were prepared for controlled IGF-I release in the context of cartilage repair . Chondrogenic differentiation of human bone marrow-derived mesenchymal stem cells (hMSCs) was observed, starting after 2 weeks and more strongly after 3 weeks . Tropical tasar silkworm *Antheraea mylitta* silk-based 3D matrices were also evaluated for *in-vitro* drug release and for the study of cell-surface interactions . The silk-based matrices contained two different model compounds, bovine serum albumin (66 kDa) and FITC-inulin (3.9 kDa), to characterize release profiles . Silk fibroin protein blended calcium alginate beads resulted in prolonged drug release without initial burst for 35 days as compared to calcium alginate beads without silk fibroin . Additionally, silk-based micromolded matrices supported a significant enhancement in cell attachment, spreading, mitochondrial activity, and proliferation with feline fibroblasts in comparison to polystyrene plates as controls . These studies indicate the potential use of slow degrading silk fibroin 3D scaffolds and tubes loaded with bioactive molecules such as BMP, HRP, and adenosine for drug-releasing biomaterials.[35]

Films

Silk films have been used with covalent decoration of functional peptides as implants for bone formation and drug delivery. For bone regeneration, BMP-2, RGD, and parathyroid hormone (PTH) can be directly immobilized on silk films using carbodiimide chemistry . Differentiation of human bone marrow-derived stem cells cultured with the decorated silk films was induced by immobilized BMP-2. Also, the utility of silk films to promote long-term adenosine release from adenosine kinase deficient embryonic stem cells has been investigated . These studies demonstrated that silk fibroin constitutes a suitable material for the directed differentiation of embryonic stem cells and for cell-mediated therapeutic release of adenosine. Silk films decorated with bioactive molecules could therefore be used for local drug delivery via direct implantation.[36][37]

Silk films have also been used to promote stabilization of entrained molecules such as enzymes or therapeutic proteins . Glucose oxidase, lipase, and HRP were entrapped in silk films over 10 months and significant activity if the enzymes were retained, even when stored at 37°C. Silk films result in stabilization of enzymes without the need for cryoprotectants, emulsifiers, covalent immobilization or other techniques. Further, the stabilization of enzymes in silk films is amenable to environmental distribution without refrigeration, and offers potential use *in-vivo* such as the delivery of bioactive molecules.[38]

Nanofibers

Scaffolds for tissue engineering may mimic the structure and biological function of the extracellular matrix. The natural extracellular matrix is a composite material with fibrous collagens embedded in proteoglycans. The collagen fibers are organized in a 3D porous network that form hierarchical structures from nanometer length scale multi-fibrils to macroscopic tissue architectures . The structures generated by electrospinning contain nanoscale fibers with microscale interconnected pores, resembling the topographic features of the extracellular matrix. Therefore, silk fibroin fiber scaffolds formed by electrospinning have potential as scaffolds. Silk fibroin fiber scaffolds containing BMP-2 and/or nanoparticles of hydroxyapatite (HAP) prepared via electrospinning have been studied for *in-vitro* bone formation from hMSCs . The bioactivity of BMP-2 was retained after the aqueous-based electrospinning process, and the nanofibrous electrospun scaffolds with co-processed BMP-2 supported high calcium deposition and enhanced transcript levels of bone-specific markers, indicating that the electrospun scaffolds were an efficient delivery system for HAP nanoparticles and BMP-2.[39]

Nanoparticles

Drug delivery systems via silkworm silk-based nanoparticles have been investigated. Biologically derived silk fibroin-based nanoparticles (<100 nm) for local and sustained therapeutic curcumin delivery to cancer cells were fabricated by blending with noncovalent interactions to encapsulate curcumin in various proportions with pure silk fibroin or silk fibroin with chitosan. Silk nanoparticles from silk fibroin solutions of

domesticated *B. mori* and tropical tasar silkworm *A. mylitta* were stable, spherical, negatively charged, 150-170 nm in average diameter and showed no toxicity . The silk nanoparticles were observed in the cytosol of murine squamous cell carcinoma cells, and the growth factor release from the nanoparticles showed significantly sustained release over 3 weeks, implying potential application as a growth factor delivery system .The silk-based nanoparticles containing curcumin showed a higher efficiency against breast cancer cells and have potential to treat *in-vivo* breast tumors by local, sustained, and long-term therapeutic delivery. Silk sericin-poloxamer nanoparticles loaded with both hydrophilic and hydrophobic drugs were reported to be stable in aqueous solution, small size (100-110 nm) and rapidly taken up by cells[40][41]

Microspheres

Silk fibroin microspheres were processed using spray-drying, however, the sizes of the microspheres were above 100 μm, which is suboptimal for drug delivery. Other methods to prepare silk microspheres include lipid vesicles as templates to efficiently load bioactive molecules for local controlled releases was reported recently. The lipid is subsequently removed by methanol or NaCl, resulting in silk microspheres consisting beta-sheet structure and approximately 2 μm in diameter [42]. The silk microspheres loaded with HRP, used as a model drug, demonstrated controlled and sustained release of active enzyme over 10-15 days. Growth factor delivery via the silk microspheres in alginate gels was also reported to be more efficient in delivering BMP-2 than insulin-like growth factors, probably due to the sustained release of the growth factor . Additionally, growth factors successfully formed linear concentration gradients in scaffolds to control osteogenic and chondrogenic differentiation of hMSCs during culture. This silk microsphere/polymeric scaffold system is an option for the delivery of multiple growth factors with spatial control for *in-vitro* and *in-vivo* 3D cultures. In more recent studies, a new mode to generate micro- and nanoparticles from silk, based on blending with polyvinyl alcohol was reported . This method simplifies the overall process compared with lipid templating and provides high yield and good control over the feature sizes, from 300 nm to 20 μm, depending on the ratio of polyvinyl alcohol/silk used [43].

Coatings

There is a critical need in medicine to develop simple and versatile methods to assemble robust, biocompatible, and functional biomaterial coatings that direct cell outcomes. Coatings of silk fibroin have been studied to provide interfaces for biomaterials [30][31]. The driving force of self-assembly to form coatings is hydrophobic and some electrostatic interactions. The flexibility of silk-based coatings has been investigated using an aqueous stepwise deposition process with *B. mori* silk solution, which can control the structure and stability of the silk fibroin in layer-by-layer films . The thickness of one layer was reported to be around 10 nm when deposited from a 1 mg/mL silk aqueous solution. The secondary structure of silk fibroin in the coatings was regulated to control the biodegradation rate, which indicates

that release of drugs from these coatings can be controlled via layer thickness, numbers of layers and secondary structure of the layers. The silk coatings have also been formed on poly(lactide-*co*-glycolic acid) (PLGA) and alginate microspheres for protein delivery . The silk coatings on PLGA microspheres was reported to be ~1 μm and discontinuous, while those on alginate microspheres was ~10 μm thick and continuous. These coatings provide mechanically stable shells as well as a diffusion barrier to the encapsulated protein drugs . Nanolayer coatings of silk fibroin to contain model compounds of small molecule drugs and therapeutically relevant proteins, such as rhodamine B and azoalbumin, have been prepared using the stepwise deposition method . Multilayered silk-based coatings have been developed and used as drug carriers and delivery systems to evaluate vascular cell responses to heparin, paclitaxel, and clopidogrel . Cell attachment and viability with human aortic endothelial cells and human coronary artery smooth muscle cells on the drug-incorporated silk coatings demonstrated that paclitaxel and clopidogrel inhibited smooth muscle cell proliferation and retarded endothelial cell proliferation [44][45]. The silk multilayers with heparin promoted human aortic endothelial cell proliferation while inhibiting human coronary artery smooth muscle cell proliferation, which was a desired outcome for the prevention of restenosis . Solid adenosine powder coated with silk fibroin were investigated for local and sustained delivery of the anticonvulsant adenosine from encapsulated reservoirs. Reservoir coating thickness was varied through manipulation of the silk coating solution concentration and the number of coatings applied. An increase in either coating thickness or crystallinity delayed adenosine burst, decreased average daily release rate, and increased the duration of release[46].

RECENT ADVANCEMENT AND FUTURE PROSPECTIVES

Silk-based biomaterials to deliver bioactive molecules, such as small drugs, proteins, and genes, are described in this review. The remarkable mechanical properties, versatile processing in an aqueous environment, biocompatibility, and controlled degradation suggest silks (both native as well as recombinant) are attractive biomaterials for controlled and sustained release, stabilization and delivery of bioactive molecules. Silk solutions can be morphed into a variety of biomaterial formats, including films, 3D porous scaffolds, hydrogels, micro- and nano-spheres, nanofibers, and coatings. The degradation rate of these biomaterials can be also controlled during processing, by the secondary structures. In addition to these useful properties, silk proteins derived from recombinant DNA technology can be bioengineered for highly tailored chemistries, greatly expanding the suite of options for targeted delivery. Targeted-delivery function is a significant factors in drug delivery, hence, these silk proteins can be prepared with functional sequences to hone to specific cells, tissues or organs, as a useful strategy for silk-based delivery systems with bioactive molecules. When combined with the novel features of the silk proteins themselves, including self-assembly, robust mechanical properties, water-based processing, controlled biodegradation and biocompatibility,

silks offer a unique and versatile delivery platform for small molecules, large proteins, DNA and RNA. Hybrid or composite silk-based materials containing other biopolymers, have not been extensively studied, yet should provide applicable mechanical, thermal, and biological properties for not only drug/gene delivery but also for tissue engineering, medical imaging, and regenerative medicine.

Silk Microneedles for Transdermal Drug Delivery of Wide Range of Compounds

Silk fibroin is an excellent biocompatible, biodegradable material that maintains the chemical properties of embedded substances. Because the process of growing these microneedles is done at standard temperature and pressure, the chemicals that are drawn into them remain perfectly preserved for delivery.The rate at which compounds are released and the microneedle matrix biodegrades can be controlled at a later stage of the process. Silk microneedles loaded with tetracycline were found to inhibit the growth of *Staphylococcus aureus*, demonstrating the potential of the microneedles to prevent local infections while also delivering therapeutics[47]

Silk fibroin as a vehicle for drug delivery applications

Silk fibroin (SF), a naturally occurring protein polymer, has several unique properties making it a favorable matrix for the incorporation and delivery of a range of therapeutic agents. SF is biocompatible, slowly biodegradable, and endowed with excellent mechanical properties and processability. Novel manufacturing techniques including mild all-aqueous processes have expanded its range of application even to sensitive protein and nucleic acid therapeutics. SF matrices were demonstrated to successfully deliver protein drugs and preserve their potency. Adjustments in SF crystallinity, concentration and structure, the design of the delivery systems as well as the molecular weight and structure of the embedded agents represent important variables when it comes to precisely tailor the release kinetics of SF matrices. Other strategies to fine-tune the release from SF matrices comprise the embedment of drug loaded micro- or nanoparticles or the coating of micro- or nanoparticles with SF films. So far, the main focus of SF drug delivery systems has been on tissue regeneration applications. For instance, growth factor loaded SF scaffolds were suggested for the tissue engineering of bone and cartilage, as well as for vascular and nerve regeneration devices and wound healing products. Moreover, SF matrices were proposed for oral, transmucosal and ocular drug delivery.[48]

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