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SILK FIBROIN: A SMART BIOMATERIAL FOR LONG TERM AND TARGETED NANOTHERAPEUTICS

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Abstract:
With the advancement of chemical and polymer sciences, the various strategies have been grown up for the development of novel Nanotherapeutics. Controlled Release Drug Delivery strategy has also been utilized by the novel Nanoformulations to alter the pharmacokinetic and pharmacodynamic property of drugs as well as to target the drug at different sites inside the body. Drug carrier materials play a significant role in the delivery of drugs. These carriers are processed into different drug controlled release systems. As the time passed the preference for biodegradable biomaterials have been increasing day by day. The biomaterial is those substances that have been engineered to interact with biological systems for therapeutic or diagnostic purposes. Biomaterials are mainly derived from natural materials but are can also be synthesized in the laboratory. Silk fibroin (SF) is a natural protein polymer that has been approved as a biomaterial by the US Food and Drug Administration (FDA). Silk fibroin based materials have a great biocompatibility profile due to their cytocompatibility and relatively lower or similar immunogenic potential compared to other common degradable polymers. SF has many unique characteristics, including appropriate mechanical properties, versatile process ability in an aqueous environment, biocompatibility, and a controlled degradation rate that make it an excellent candidate for drug delivery applications. So far silk-based nanoparticles have been developed to target lung, tumor and ocular disease for the delivery of proteins, small molecules, and anticancer drugs.
Key Words: Controlled Release Drug Delivery Systems, Nanoparticle, Biodegradable polymer, tumour.

Introduction

Controlled release is the term that refers to the presentation or delivery of compounds in response to stimuli or time. Controlled Release Drug Delivery Systems (CRDDS) are those dosage forms from which the release rate of the drug is controlled, either with respect to time (temporal) or related to site (spatial). Prolonging the duration of action of the drug at a predetermined rate, localizing the drug action by spatial placement of the controlled release system and targeting drug action by using carriers or chemical derivatization to deliver drugs to a particular target are the main objectives of a controlled

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release drug delivery system. An ideal Controlled drug delivery system is the one, which delivers the drugs at a predetermined rate, locally or systematically, for a specific period of time (Allen and Cullis 2004; Bhowmik *et al* 2012; Park 2014; Uhrich *et al* 1999).

The main rationale behind the development of CRDDS is to alter the pharmacokinetics and pharmacodynamics of drugs by using novel molecular structure and/or physiological parameters inherent in selected route of administration. There are mainly three types of controlled release therapeutic systems available, viz. passive programmed, active programmed and active self-programmed. Most of the rate controlled release delivery systems fall under the category of passive programmed, in which the drug release rate is predetermined and remains unaltered irrespective of the external physiological environment. Active programmed systems are those, in which the release rate drug can be altered by a source external to the body, e.g. insulin metered pumps. The active, self-programmed system control the release rate of the drug in response to information, by a sensor, or by changing biological environment systems, e.g. releasing a higher quantity of insulin in response to a higher blood glucose level in diabetes (Huang and Brazel 2001; Jantzen and Robinson 2002; Sershen and West 2002).

There are several advantages of CRDDS over conventional dosage forms. These novel delivery systems help to maintain a steady plasma level of the drug, ideally within the therapeutic window of the drug over a prolonged time period, which simulates an intravenous infusion of a drug. This eventually reduces the frequency of drug administration and hence enhances patient compliance. The total cost of therapy of the controlled release product could be comparable or lower than the immediate release product with a reduction in side effects. The overall expense in disease management also would be reduced. This greatly reduces the possibility of side effects, as the scale of side effects increases as we approach the maximum safe concentration (Dash *et al* 2010; Gelperina *et al* 2005; Kost and Langer 2012).

Though CRDDS has various advantages, it has some disadvantages too. The drug content of CRDDS is normally higher as compared to conventional dosage forms. Due to the improper formulation, sometimes a relatively large quantity of drug in a controlled release formulation may be rapidly released, introducing a potentially toxic quantity of the drug into systemic circulation. This phenomenon is known as Dose dumping. Dose dumping can lead to fatalities in case of potent drugs, which have a narrow therapeutic index. With conventional dosage forms, dose adjustment is simple. For example, a tablet can be divided into two fractions. But in the case of a controlled release dosage form, controlled release property may get lost, if the dosage form is fractured. Hence with CRDDS, dose

adjustment is not that easy. 'Absorption window' becomes important for CRDDS and may give rise to unsatisfactory drug absorption in-vivo despite excellent in-vitro release characteristics. As the lesser amount of drug is released from the CRDDS as compared to conventional dosage forms, there is a higher chance of losing the drug in blood by first pass metabolism. Because, with higher drug concentration, hepatic enzymes get saturated and hence some drugs can escape those enzymes. But when the concentration is low, achieving an enzyme-saturating concentration is not possible. Over that, controlled release medication does not permit prompt termination of therapy. Immediate changes in drug levels during therapy, such as might be encountered if significant adverse effects are noted, cannot be accommodated (Bajpai *et al* 2008; Ratilal *et al* 2011).

CRDDSs are applied for different purposes, it may be simply coating a tablet with enteric material to bypass the gastric acidic environment or more complicated delivery of cytotoxic drugs to tumours, delivery of genetic materials to target cells, delivery of drugs to brain through blood-brain barrier (BBB) for treatment of brain diseases etc. (Elzoghby, Samy and Elgindy 2012; Slowing *et al* 2008).

The applications of CRDDS can be classified into mainly three classes.

i. **Extended release dosage forms:** These dosage forms allow at least twofold reduction in dosage frequency as compared to that drug presented as an immediate or conventional release dosage form (Mansour *et al* 2010; Shen and Burgess 2012; Tajiri *et al* 2010).
ii. **Delayed release dosage forms:** These dosage forms release a discrete portion or portions of the drug at a time or times other than promptly after administration although one portion may be released promptly after administration (Farinha *et al* 2000; Guarascio and Slain 2015).
iii. **Targeted release dosage forms:** These are the dosage forms that releases the drug at or near the intended physiological site of action. These formulations may have either immediate or extended release characteristics. In recent times, targeted release dosage forms have got the most attention from formulation scientists as it has the potential to treat specifically the affected tissue or cells by delivering highly potent drugs to the affected area while rest of the body remaining unexposed to the drug. Different types of formulations (e.g. nanoparticles, liposomes, niosomes, solid lipid nanoparticles, dendrimers, nanostructured lipid carriers etc.) with different types of materials (e.g. polymers, proteins, lipids etc.) has been formulated to deliver drugs to different body parts or organs. In this review, we will discuss the targeting

efficiency of nanoparticles formulated from silk fibroin (SF) for different body parts (Uhrich *et al* 1999; Varde and Pack 2004).

The biomaterial is those substances that have been engineered to interact with biological systems for some medical purposes; either for a therapeutic purpose, i.e. to treat, augment, repair or replace a tissue function of the body, or for a diagnostic purpose. Biomaterials are mainly derived from natural materials but are can also be synthesized in the laboratory from metallic components, polymers, ceramics or composite materials using a variety of chemical approaches. Biomaterials are often used for a medical application, and thus it comprises the whole or part of a living structure or a biomedical device which performs, augments, or replaces a natural function. The function may be of benign type meaning they don't undergo any changes with time, like being used for a heart valve; or may be of the bioactive type with a more interactive functionality, which gradually becomes a part of the physiological system, such as hydroxyapatite-coated hip implants. Biomaterials are widely used in dental applications, surgery, and drug delivery. Drug delivery systems formulated with biomaterials permits the prolonged release of a drug over an extended period. The biomaterial can also be used as an autograft, allograft or xenograft used as a transplant material (Davis 2003; Peppas 2000; Vepari and Kaplan 2007).

The major advantages of natural biomaterials are that they are available in large quantities constantly at a reasonable price, they already have binding sites for cells and adhesion molecules so the biocompatibility is not an issue with natural biomaterials. As most of the natural products have some disadvantages, natural biomaterials to have some. Due to natural variability in the in vivo source, the lot-to-lot variability is always a concern. Additionally, potential impurities may also result in unwanted immune reactions. They have limitations in their mechanical properties (Ige, Umoru and Aribo 2012; Zhang 2002).

The main advantages of synthetic biomaterials over natural biomaterials are their high reproducibility, availability on demand and constant quality supporting industrial-scale production. Moreover, by application of slight changes of production, an easy control of mechanical properties, degradation rate, shape, composition, etc. can be adjusted to current needs. However, synthetic materials often lack sites for cell adhesion and the biocompatibility is frequently questionable. Biocompatibility and support of stem cell differentiation are not clear either, while immune reactions are also possible (Khaing and Schmidt 2012; Nair and Laurencin 2007). A brief comparison between natural and synthetic biomaterial is given in Table 1.

Review of research papers of the last 15 years shows that alkali-heat purified silk fibroin-based materials has a great biocompatibility profile due to their cytocompatibility and relatively lower or similar immunogenic potential compared to other common degradable polymers, such as collagen and PLGA. *In vitro* cultures of fibroin with various cell types,

Table 1: Comparison of natural and synthetic biomaterials.

Properties	**Natural Biomaterial**	**Synthetic Biomaterial**
Immunogenicity	Maybe immunogenic if not processed properly.	Though less, Immune reactions are possible.
Compatibility	Biocompatible	Not clear
Biodegradability	Biodegradable	Non-biodegradable
Availability	Limited	Available as per demand
Mechanical Strength	Poor	High
Use	Drug delivery systems, tissue engineering.	In dental surgery, bone replacement.
Examples	**Proteins:** Collagen, Fibrin, Silk. **Polysaccharides:** Agarose, Alginate, Hyaluronic acid, Chitosan.	**Organic:** PGA, PLA, PLGA, PEG, Peptides. **Inorganic:** Ceramic, Metal, Hydroxyapatite.

including fibroblasts, keratinocytes, hepatocytes, osteoblasts, epithelial, endothelial, glial, and mesenchymal stem cells indicate desirable cytocompatibility profiles for various silk fibroin. Transient pro-inflammatory (IL-1β) and inflammatory (COX-2) gene expression in response to stimulation with fibroin films is similar to those of collagen or PLA polymer matrices. Furthermore, fibroin films show higher cell proliferation rate as compared to collagen or PLA, highlighting the relatively low immunogenic potential and high cytocompatibility of silk fibroin. Inflammatory potential of different components of *Bombyx mori* silk, including silk fibers including sericin-rich surface coating layer, alkali-heat regenerated fibroin fibers, alkali-heat degumming supernatant rich in sericin, regenerated fibroin fibers incubated in the alkali-heat degumming supernatant, and insoluble fibroin particles obtained by chymotrypsin proteolysis of degummed fibroin was studied. The results indicate that stimulation of macrophages with none of the individual silkworm silk components produced elevated levels of pro-inflammatory TNF either in short (1-day) or long-term (7-day) cultures (Jin 2004; Merkle *et al* 2006; A Motta *et al* 2004; Panilaitis *et al* 2003; Santin *et al* 1999).

Molecular and physical properties of SF

Silk fibroin is a natural protein polymer that has been approved as a biomaterial by the US Food and Drug Administration (FDA). Silk proteins collected from different silkworm species exhibit variations in their structure and properties (Craig *et al* 1999; Fu, Shao and Fritz 2009; Sutherland *et al* 2010). Silk proteins can be isolated either from the cocoons or the silk glands of silkworms (Kundu *et al* 2014; Rockwood *et al* 2011).

Silk fibroin is being used as a material great potential to formulate drug delivery systems in the recent past. Different physicochemical properties of Silk fibroin can affect the drug loading as well as releasing pattern from the formulations. Some of the properties which play crucial roles with this perspective are described below.

i. Arrangement of amino acids and their derivatization in silk fibroin

Silk obtained from the cocoon of domesticated silkworm, *Bombyx mori* is one of the best-characterized silk. Silk from *Bombyx mori* is made of two structural proteins, the fibroin heavy chain (~325 kDa) and light chain (~25 kDa). Glue-like protein sericin (20 kDa to 310 kDa) hold the chains together. Sericin has been associated with immune response, but due to their higher hydrophilicity as compared to fibroin they can be easily removed by boiling silk in alkaline solutions (Altman *et al* 2003). The heavy chain of silk fibroin contains alternating hydrophobic and hydrophilic blocks similar to those seen in amphiphilic block copolymers. The hydrophobic blocks consist of highly conserved sequence repeats of GAGAGS and less conserved repeats of GAGAGX (where X is either V or Y) that make up the crystalline regions of silk fibroin by folding into intermolecular β-sheets. The hydrophilic part of the core is non-repetitive and very short compared to the size of the hydrophobic repeats (Bini, Knight and Kaplan 2004).

Due to its amino acid sequence, silk fibroin provides opportunities for chemical modification. Amines, alcohols, phenols, carboxyl groups, and thiols have been explored as potentially reactive side groups for the chemical modification of silk fibroin. For instance, carboxylic acid side groups from aspartic and glutamic acids, representing 2–3% of the total amino acid content of silk fibroin, have been derivatized with primary amines of peptides such as the RGD sequence, with the aim to improve cell adhesion (Sofia *et al* 2001). To expand the range of functionalization, tyrosine residues, representing 10% of the total amino acid content of silk fibroin, were modified with a variety of functional groups (Zhou *et al* 2001). Such modifications led to changes in silk fibroin hydrophilicity and charge and are, therefore, expected to alter the interaction

between drug molecules and silk fibroin. It is envisioned that by introducing distinct functional groups into silk fibroin and by varying the degree of functionalization per silk fibroin molecule, a variety of drugs in different amounts can be loaded and released with distinct kinetics, providing a wide range of adjustable drug release systems. In addition to drug delivery contributions, functionalization may also promote cell adhesion and spread or address cellular signalling pathways through specific cell–matrix interactions (Murphy, John and Kaplan 2008).

ii. Chain length & Molecular weight of silk fibroin

The molecular weight of a polymer strongly influences its mechanical properties and biodegradability, and, therefore, its field of application in drug delivery. For this reason, customized polyesters such as poly (lactide-co-glycolide) (PLGA) of various lactide: glycolide ratios have been widely used for drug delivery applications (Park 1994; Wenk *et al* 2009; Zilberman and Grinberg 2007). Natural silks exhibit large molecular weights due to the long amino acid chains. Lower molecular weights of silk fibroin can be obtained by processing silk. Prolonged boiling of silk cocoons in Na_2CO_3 solutions is a common method to remove sericin from fibroin, was shown to cause extensive hydrolytic degradation of the silk fibroin protein. As this method is not well controlled, hence might produce a wide molecular weight distribution. Silk fibroin with diverse molecular weights may be also obtained by adding sodium hydroxide to aqueous silk fibroin solutions at different processing temperatures (Cai *et al* 2002) or by enzymatic degradation. The best approach to produce silk fibroin analogues with distinct molecular weights is genetic engineering (Megeed, Cappello and Ghandehari 2002). It has to be considered that silk fibroin analogues with much lower molecular weights may have compromised mechanical properties. Thus, the application of such analogues to load-bearing body sites must be further explored. From the above discussion it can be concluded that variations in the processing of silk fibroin or its genetic engineering can produce silk fibroin with different molecular weights, which may influence bulk viscosity, further processing into drug delivery systems, bulk crystallinity, degradation rates and, thereby, the release kinetics of embedded drugs (Asakura *et al* 1985; Zuo, Dai and Wu 2006).

iii. Solubility of silk fibroin

Crystalline silk fibroin is insoluble in most solvents that are widely used to dissolve polymers typical for drug delivery applications including water. Silk fibroin is commonly dissolved in highly concentrated salt solutions of lithium bromide, lithium thiocyanate, calcium thiocyanate or calcium chloride (Hu *et al* 2012). Such electrolyte solutions are

able to disrupt the hydrogen bonds that stabilize β-sheets (Phillips *et al* 2004). Dialysis against water or buffers is performed to remove the electrolytes. The resulting aqueous silk fibroin solutions can then be freeze-dried, followed by dissolution of the dry silk fibroin in hexafluoroisopropanol (HFIP). Such solutions may be further processed into the desired drug delivery device (Karageorgiou *et al* 2004a; Kirker-Head *et al* 2007). However, HFIP is an expensive and toxic solvent. Moreover, in contact with sensitive biologicals, e.g. growth factors, HFIP may exert detrimental effects on protein folding and biological potency. The alternative use of aqueous silk fibroin solutions offers more gentle processing conditions to fabricate drug delivery systems for such biologicals. Interestingly, the processing of aqueous instead of HFIP solutions of silk fibroin offers several advantages like the option to load silk fibroin based constructs with drugs, micro-particles that are insoluble in aqueous solutions (Wenk *et al* 2009). Further advantages of processing aqueous instead of HFIP solutions of silk fibroin are the ease of sterilization by filtration and the absence of residual solvents in the fabricated matrix.

A common problem with the processing of aqueous silk fibroin solutions still exists, namely the premature re-precipitation into its water insoluble, β-sheet enriched silk II state. Especially highly concentrated silk fibroin solutions tend to aggregate in a matter of hours to days due to inter and intramolecular interactions of the protein. Various approaches that prevent the formation of the β-sheet structure were studied in order to maintain higher silk fibroin concentrations in a soluble state. For instance, phosphorylation of genetically engineered silk has been shown to increase the overall aqueous solubility of the protein through a combination of steric hindrance and charge (Winkler, Wilson and Kaplan 2000). Recently, the modification of the tyrosine residues in silk fibroin by a diazonium coupling reaction with 4-sulfanilic acid led to a sulfonated silk fibroin derivative that demonstrated to inhibit spontaneous protein aggregation or gelation for more than one year, whereas unmodified silk fibroin was found to gel within one month (Murphy, John and Kaplan 2008). Nevertheless, the sulfonated silk fibroin derivative could still transform into a β- sheet enriched structure when treated with methanol. Periodic interruption of a silk-like polymer (SLP) featuring (GAGAS)n blocks with the elastin-like sequence (GVGVP)n to produce genetically engineered silk-elastin-like polymers (SELPs) was also found to increase the solubility while decreasing the β-sheet based crystallinity (Cappello *et al* 1990). The possibility to control the solubility of silk fibroin not only allows for longer storage times for silk fibroin solutions but also for an increase in silk fibroin concentration without aggregation. As a consequence, thereof, it is possible to fabricate silk fibroin matrices with different characteristics than matrices

prepared from solutions with lower silk fibroin concentration, e.g., matrices with denser structures which may influence release kinetics.

iv. Hydrophobicity & Crystallinity of silk fibroin

The hydrophobic blocks of silk fibroin make up the crystalline regions of silk fibroin by their capacity to form intermolecular β-sheets. Different treatments dehydrate and destabilize the random coil and convert the silk fibroin from a more unstable conformation (silk I) to a different conformation (silk II), which is more stable and characterized by an increase in β-sheet content. The most common method to enrich silk fibroin in β-sheet structure and thus induce water insolubility is a treatment with methanol (Asakura *et al* 1985; Monti *et al* 1998). High temperatures (Antonella Motta, Fambri and Migliaresi 2002), a pH close to the isoelectric point of silk fibroin (around 4), the use of salts (Dicko *et al* 2004; Zong *et al* 2004) and shear-force (Jin and Kaplan 2003; Xie *et al* 2006) can also be applied to increase its β-sheet content. However, when sensitive molecules such as growth factors need to be incorporated into silk fibroin, milder conditions to evoke β-sheets are preferred. Exposure to water vapor has been demonstrated to be such a mild alternative to induce water insolubility (Min *et al* 2006; Wenk *et al* 2008). Moreover, slow freezing rates, being a consequence of higher freezing temperatures (above −20 °C), resulted in an increase in β-sheet based crystallinity (Li *et al* 2001; Nam and Park 2001).

Recently, a time-dependent increase in the β-sheet structure was observed when treating silk fibroin micro-particles with a saturated NaCl solution, obtaining β-sheet contents of about 34%, 51%, and 67% when treating for 1 h, 4 h, and 15 h, respectively. The β-sheet content of silk fibroin micro-particles treated with methanol for 30min was analyzed to be 58%, comparable to longer treatments with NaCl (Wang *et al* 2007). Additionally, the inclusion of elastin-like domains in silk fibroin could reduce crystallinity (Cappello *et al* 1990; Megeed, Cappello and Ghandehari 2002). Alterations in both crystallinity and hydrophobicity offer options to affect the interaction between drug molecules and silk fibroin. Moreover, a change in crystallinity influences the degradation rate of silk fibroin, which could be an attractive approach towards drug delivery systems with distinct release kinetics.

v. Swelling properties of silk fibroin

The release of drugs from polymer matrices depends partially on the degree of swelling, which in turn depends on the ionization of the polymer network, its degree of crosslinking and its hydrophilic/hydrophobic balance (Peppas and Khare 1993).

Changes in polymer compositions can influence the degree of swelling. For instance, an increase in the length of elastin repeating units in the backbone of SELP hydrogels, while keeping the length of silk repeating units constant can result in an increased degree of swelling due to a decrease in cross-linking density (Haider *et al* 2005). This can potentially increase the cumulative amount and rate of drug release. The swelling ratio of silk fibroin scaffolds has also been shown to decrease with an increase in silk fibroin concentration. The blending of silk fibroin with other materials such as chitosan, hyaluronic acid, and chitosan led to increased swelling when compared with plain silk fibroin (Garcia-Fuentes *et al* 2008; Gobin, Froude and Mathur 2005; Rujiravanit *et al* 2003).

vi. Biocompatibility of silk fibroin

The foreign body response following implantation of silk fibroin *in vivo* has been described to be comparable to or even less than the most popular materials in use today as biomaterials (Altman *et al* 2003; Meinel *et al* 2005). All-aqueous- and HFIP-derived scaffolds have been tested in a one-year implantation study in rats. Those scaffolds were well tolerated by the host animals, and the host immune response to the implanted scaffolds was low and local (Wang *et al* 2008), consistent with another study with silk fibroin films (Meinel *et al* 2005).

vii. Degradation and biodegradation of silk fibroin

The biodegradation rate of a drug delivery system for tissue regeneration should be adjustable to kinetically match the evolving environment during healing and regeneration (Lanza, Langer and Vacanti 2014). The degradation of many commonly used polymers in drug delivery is fast (Langer and Vacanti 1993), typically in the range of weeks or months as with many poly(lactide-co-glycolide) (PLGA) copolymers. This is a disadvantage when the integrity of a load-bearing system has to be preserved. Moreover, by hydrolytic degradation some polymers such as the widely used PLGA generate acidic moieties, resulting in a local decrease in pH (Fu *et al* 2000), which may cause an inflammatory response both in the implant and the adjacent tissue (Zolnik and Burgess 2008) and initiate acid catalyzed protein degradation (Houchin and Topp 2008). It was demonstrated in a recent study that the hydrolytic degradation of water vapor treated silk fibroin scaffolds *in vitro* without the presence of proteolytic enzymes is minor, with only about 4% mass loss within 7 weeks (Wenk *et al* 2009). Being a protein, biodegradation of silk fibroin predominantly occurs through proteolytic enzymes, with non-toxic degradation products and unproblematic metabolization *in vivo*. The

implantation site, the mechanical environment and the size and morphology of the drug delivery device are likely to affect the degradation rate *in vivo*. The biodegradation of silk fibroin has been described to directly relate to its β-sheet content. For instance, the annealing of silk fibroin films with water is known to induce conformational transition. When incubated *in vitro* in the presence of a protease, the mass loss of such water-annealed silk fibroin films, typically having a lower β-sheet content due to the slow annealing process, was more pronounced than that of methanol-treated films showing predominantly silk II conformation (Jin *et al* 2005). Furthermore, silk fibroin scaffolds have been prepared by adding NaCl particles to either silk fibroin aqueous solutions (all-aqueous-derived) or to silk fibroin dissolved in HFIP (HFIP-derived) to induce conformational transition (Wang *et al* 2008). After fabrication, the NaCl particles were leached out in water. The biodegradation of such prepared 3D porous scaffolds was studied in vivo in rats. Most all aqueous- derived scaffolds were completely degraded after 6 months, whereas HFIP-derived scaffolds still existed showing varying degrees of biodegradation. The total β-sheet content was not significantly different between all-aqueous- and HFIP-derived scaffolds, but the size, distribution, and nature of the crystals may vary, contributing to the different biodegradation rates, along with different structural, morphological and surface characteristics. Scaffolds prepared from solutions of higher silk fibroin concentration degraded more slowly than those made from solutions of lower concentrations (Wang *et al* 2008), which may be explained by the greater extent of material that has to be hydrolysed and the greater mechanical strength of the scaffolds with increasing silk fibroin concentration (Kim *et al* 2005). The experiments demonstrate that short-term drug delivery systems can be best matched by systems prepared by an all-aqueous process or water annealing due to its faster degradation time while long-term delivery devices can be better met by slower degrading HFIP-derived systems or methanol-treated devices. On the other hand, previous reports on SELPs have shown that their *in vivo* degradation can be controlled by varying the composition and sequence of the polymers. Using a protein engineering approach, biodegradation rates in response to tissue-specific enzymes may be tailored by incorporating protease specific cleavage sites into silk fibroin.

viii. Thermal Stability of silk fibroin

To have a stable drug delivery device, the stability of the formulation polymer is very important. Untreated silk fibroin matrices are low in β-sheet content which is more hygroscopic and thus highly sensitive to humidity. Incubation at high humidity has been shown to change the conformational state of silk fibroin, leading to increased β-sheet

contents (Min *et al* 2006; Wenk *et al* 2008). No systematic investigations exist on the storage stability of silk fibroin matrices. Silk fibroin displays an exceptional thermal stability and remains unaffected up to a temperature of 140 °C. The glass transition temperature (Tg) of proteins is considered to be a major determinant of protein self-assembly. Dry silk fibroin films prepared from silk taken from the posterior part of the middle division of the silk gland of the silkworm *Bombyx mori* demonstrated a Tg of approximately 175 °C, above which there is free molecular movement to transform into the stable β-sheet conformation, showing stability up to around 250 °C. The Tg of frozen silk fibroin solution was reported to be in the range of about −34 to −20 °C (Li *et al* 2001). The higher the pre-freezing temperature above the glass transition, the longer the time needed for ice crystals to form and grow in size. This affects the pore size within silk fibroin matrices, leading to bigger pores, as well as an increase in crystallinity.

ix. Mechanical properties of silk fibroin

Many scaffolds prepared from polymers including PLGA and collagen lack sufficient mechanical strength for load-bearing purposes (Holland and Mikos 2006). Enhanced mechanical strength, however, is an important issue when a drug delivery device is also used as a scaffold with load-bearing function, as frequently needed in bone repair. For instance, the physical properties of collagen are rather limited unless crosslinked. However, crosslinking reactions may have adverse effects on adjacent native tissue such as cellular toxicity and inflammatory responses (Bhrany *et al* 2008; Lee and Shin 2007; Schmidt and Baier 2000). In contrast, owing to its robust β-sheet conformation, crystalline silk fibroin exhibits outstanding mechanical properties and does not need to be crosslinked. Compared to the mechanical properties of porous biodegradable polymeric scaffolds often considered in bone-related tissue engineering studies (e.g. collagen, chitosan, and hyaluronan), water-stable aqueous-derived silk fibroin scaffolds showed favorable mechanical properties. The compressive strength and modulus of those silk fibroin scaffolds were reported to increase with increasing silk fibroin concentration, decreasing pore size and more uniform pore distribution (Kim *et al* 2005). Another study also compared the mechanical properties of three-dimensional porous scaffolds using the different polymeric material. The compressive modulus of gas foamed silk fibroin scaffolds was superior as compared to scaffolds prepared from collagen, chitosan, PLGA, and PLLA. The compressive strength and modulus of those silk fibroin scaffolds were shown to depend on the preparation method of the scaffolds. The compressive strength and modulus of gas foamed silk fibroin scaffolds were reported to be higher than of salt

leached silk fibroin scaffolds, which was explained by the uniform distribution of the pores within the gas foamed scaffolds and the smaller pore size (Li *et al* 2008).

Silk based nanoparticles

i. Drug incorporation in SF nanoparticle

The easy and common way of incorporation of drug into SF delivery system is by mixing them with the SF solution from which the fabrication of the formulation to be done prior to the processing (Lammel *et al* 2010; Yan *et al* 2009). But there is a challenge of this method, i.e. to ensure that there is no negative impact of the fabrication process on the stability and biological activity of the drug. Alternatively, the drug can be incorporated post-fabrication, such as by adsorption (Karageorgiou *et al* 2006; Kirker-Head *et al* 2007) or covalent coupling (Karageorgiou *et al* 2004b; Vepari and Kaplan 2006) of the drug to the pre-fabricated SF system. It is advantageous to bound or conjugate drug with the fabrication just prior to implantation. This would allow the SF matrix to be prepared in advance and stored stably under appropriate conditions for extended periods of times. The drug would be introduced just prior to use such that its stability and biological potency would be best maintained. Additionally, the drug would not be exposed to harsh fabrication conditions or leach out during fabrication. The capacity of a drug to bind to or form a conjugate with an SF matrix depends on its physicochemical properties and could be rather limited. For instance, owing to the mainly negatively charged side chains of the hydrophilic spacers of its heavy chain (Foo *et al* 2006; Zhou *et al* 2000). On the other hand, the main cause of interaction between SF and drugs is the interaction with hydrophobic moieties (Wenk *et al* 2008), due to the hydrophobic blocks in the heavy chain of SF. In order to improve the encapsulation and loading capacity of the drug with SF matrix, several approaches have been investigated. For instance, drug binding to SF matrices may be improved by SF modifications (Murphy, John and Kaplan 2008). The incorporation of pre-fabricated drug loaded nanoparticles into electrospun SF mats during electrospinning may be also envisioned. Again, this approach has the potential to protect the drug against harsh processing parameters, and control its release kinetics not only by retention in the SF nanofiber itself but also by the composition of the nanoparticles. The incorporation of unloaded hydroxyapatite nanoparticles into SF nanofibers upon electrospinning has been previously reported and shown promise as an osteoinductive biomaterial (Li *et al* 2006).

The covalent coupling of drugs to the SF matrix can sustain its release compared to simple adsorption. However, harsh coupling conditions may result in a loss of activity and drug.

Moreover, additional washing steps have to be included in the process in order to remove side products and additives.

Drug loading can be determined either directly by dissolution of the drug delivery device, or indirectly by measuring the drug that was not incorporated. Dissolution is preferably performed prior to inducing water insolubility. For instance, untreated SF spheres were dissolved in water to determine the content of encapsulated salicylic acid, propranolol hydrochloride and IGF-I (Wenk *et al* 2008).

ii. Control of drug release from SF nanoparticle

Suitable drug release kinetics is often a prerequisite to trigger physiological signaling with given pathological conditions. In this section, we will discuss the release of a variety of drugs from SF drug delivery devices and how their release kinetics may be controlled.

There has been a considerable amount of effort applied to determining the mechanisms of drug release from different silk-based formulations. Through a mechanistic understanding of release kinetics, critical control points in the formulation development may be elucidated to offer guidance for generating near zero-order, silk-based sustained release formulations. Based on the extensive number of applications of silk technology for drug delivery outlined above, most concluded that diffusion or a combination of diffusion, polymer swelling, and polymer degradation are the primary mechanisms governing the release of drug from silk-based formats (Bessa *et al* 2010; Hines and Kaplan 2011; Pritchard *et al* 2010; Uebersax, Merkle and Meinel 2008). The release kinetics of SF matrices was frequently studied using model drugs. Generally, the release was controlled by both the characteristics of the incorporated drug and those of the polymer matrix. In terms of the incorporated drugs, release kinetics was shown to depend on the molecular weight of the drug. As demonstrated for dextrans, an increase in molecular weight resulted in reduced release rates (Hofmann *et al* 2006). Furthermore, drug release depended on its interaction with SF. For instance, it is demonstrated that the release of propranolol hydrochloride from SF spheres was more sustained as compared to salicylic acid (Wenk *et al* 2008). This was explained by the electrostatic interaction between the positively charged Propranolol (pKa=9.5) and the negatively charged SF (pI=4.2) at the pH of the release study (pH 7.4). In contrast, repulsive forces were concluded to govern the interaction between a salicylic acid (pKa=3.0) and SF, leading to enhanced release. As to the SF matrix, higher SF concentrations resulted in lower burst and release rates. This was not only demonstrated with propranolol hydrochloride and salicylic acid encapsulated in SF spheres (Wenk *et al* 2008), but also with buprenorphine

in hydrogels (Fang *et al* 2006), possibly due to the more closely packed structure, decreased swelling and mean pore size, and/or the longer diffusional pathways. Further factors to influence the release kinetics were surface morphology, lipid content, distribution of the drug in the SF matrix, and crystallinity induced by methanol or NaCl treatment, as exemplified by the release of HRP from SF microparticles (Wang *et al* 2007). Further materials that were used to blend SF include hydroxyapatite (Du *et al* 2009), collagen (Yeo *et al* 2008), chitosan (Rujiravanit et al. 2003), PEG (Katayama, Issiki and Yoshitomi 2000), keratin (Vasconcelos, Freddi and Cavaco-Paulo 2008), hyaluronic acid (Garcia-Fuentes *et al* 2008), and poloxamer (Kang *et al* 2000), but remain yet to be tested for drug delivery applications. All of them are assumed not only to change the mechanical properties but also the release kinetics due to changes in porosity, swelling, solubility and drug–matrix interactions, thus providing a wide range of opportunities to customize the delivery properties of SF.

Microparticles and liposomes have been coated with SF in order to improve cellular recognition and prolong release. For instance, PLGA and alginate microparticles were coated by the layer-by-layer assembly technology (Wang *et al* 2007). The resulting SF coatings not only slowed down the decomposition of the alginate microparticles as compared to the corresponding uncoated microparticles but also dramatically sustained the release of HRP from PLGA microparticles and of Rh-BSA from alginate microparticles. Through diffusional hindrance, the barrier imposed by SF coatings can also result in reduced swelling. For instance, the release of emodin from 1,2-dimyristol-sn-glycero-3-phosphocholine (DMPC) liposomes was described to be the consequence of the swelling of the liposomal lamellae followed by quick diffusion of the drug. When the liposomes were coated with SF layers, the swelling of the liposomal lamellae was restricted, and emodin release was diffusion controlled and slow (Gobin *et al* 2006). Overall, the results from SF coated microparticles and liposomes suggest the development of further modifications. For instance, drugs may not only be embedded in the bulk of the particles but also incorporated into or sandwiched between the SF coatings. Layer-by-layer assembled SF coatings could be also useful for sequential release, e.g., by embedment of one drug into the microparticles or liposomes and a second or third one into the SF coating. Owing to a potential functionalization of SF, targeted drug delivery of microparticles and liposomes by coupling ligands to the SF coating could also be envisioned.

An all-aqueous, stepwise deposition process with silk fibroin protein for the assembly of nanoscale layered controlled release coatings was exploited. Model compounds,

Rhodamine B, Even Blue and Azoalbumin, representing small molecule drugs and therapeutically relevant proteins were incorporated in the nanocoating process and their loading and release behavior was quantified. Release studies in vitro showed that control of β-sheet crystal content and the multilayer structure of the silk coatings correlated with the release properties of the incorporated compounds. In particular, higher crystallinity and a thicker silk capping layer suppressed the initial burst of release and prolonged the duration of release (Wang *et al* 2007).

iii. SF nanoparticle for lung targeting

Targeted drug delivery to the lungs both for local and systemic treatment is a challenging area in pharmaceutical research, used as an alternative to oral delivery as because of more than 40% of new drugs having low bioavailability through the oral route, dose-limiting side effect and hydrophobic in nature. To provide enhanced bioavailability, reduced toxicity, perfect deposition of drug in lungs, the drug can be targeted through pulmonary route (Ravichandiran, Masilamani and Satheshkumar 2011). Every year in the united states about 220,000 individuals infected by Lung cancer which is mainly classified as non-small-cell lung carcinoma (85%) and the remaining is small-cell lung carcinoma. Platinum-based chemotherapy regimens for lung cancer administered intravenously destroy cancerous as well as normal tissues and they are riddled with many dose-limiting side effect including nephron-and cardiotoxicity, peripheral neuropathy as well as less serious symptoms of uneasiness, nausea, and fatigue. To overcome this unwanted effect nanoparticle-based drug delivery system were investigated (Babu *et al* 2013). Silk fibroin due to their biocompatibility and controlled biodegradation properties are utilized to formulate for the first time as carriers for lung drug delivery. Subia B. *et al 2014.,* investigated that silk fibroin-folate nanoparticles loaded with an anticancer drug like doxorubicin serve as promising candidates for different biomedical application in cancer research (Subia *et al* 2014). Kim *et al* 2015, investigated that cisplatin-incorporated silk fibroin nanoparticles show different release profile with or without any cross-linking agents. To enable the delivery of the airways silk fibroin particles are spray-dried or spray-freeze-dried where mannitol is added as an excipient for the stabilization of protein and for efficient dispersion using an *in vitro* aerosolization impactor. The spray-freeze-drying process is favorable for inhalation and produces higher yields of particles with high porosity. The silk-based particles show high aerosolization performance and to be cytocompatible with A_{549} human lung epithelial cell line. This *in vitro* drug release is carried out in Franz cell station which allows the mimicking of the air-liquid interface present in the lungs. It is demonstrated that cross-linked silk-based particles could

enhance cytotoxicity (Kim *et al* 2015). These novel inhalable silk-based drug carriers have the potential to be used as anticancer drug delivery systems targeted for the lungs**.** But in some cases, the product may fail to achieve its goal and to make effective delivery of drug to the targeted area researcher should have thorough knowledge in all areas like disease condition being treated, lung anatomy and physiology and the method of achieving optimum particle size, carrier suitability, and drug delivery devices.

iv. SF nanoparticle for tumor targeting

Tumour or neoplasm is an abnormal mass of tissue either solid or fluid-filled, classified into two types benign (not cancerous) and malignant (cancerous). The main aim of the researcher is to help patients by developing clinically useful formulation and targeting drug delivery to tumors which enhances the drug efficacy and minimized side effects by routinely deliver drugs in a sustained release rate. Nanoparticles using tumor-targeting drug delivery provide an opportunity for those patients who are not surgical candidates for de-bulking and which are large enough to develop vasculature (Bae and Park 2011).

Recently many researchers have worked on drug loading fibroin nanoparticles for tumor targeting which has shown potential to antitumor treatment. Incorporation of the anti-cancer drugs like paclitaxel (PTX), doxorubicin (DOX), methotrexate, curcumin, Fluxoridine, emodin etc. into SF nanoparticles has gained much interest (Zhao, Li and Xie 2015). Curcumin also known as diferuloylmethane can prevent carcinogen-induced cancer limited by its lack of water solubility and low bioavailability and, to overcome these problem multiple approaches including, nanoparticles, microparticles, liposome, micelles are being sought (Bar-Sela, Epelbaum and Schaffer 2010; Ji 2012). Lozano-Perez AA *et al*. 2015, investigated PtBz-Silk fibroin nanoparticles trigger strong cytotoxic activity against A2780 cell (human cell carcinoma), A2780 cisR (acquired resistance to cisplatin) and the triple negative breast tumor cell line MDA-MB-231, SK-BR-3 as well as MCF-7 (Lozano-Pérez *et al* 2015). Numata K *et al*. 2012, investigated nano-scale silk-based tumor homing peptides (THPs) associated with DNA are able to home specifically to tumorigenic cells. Cytotoxic activity of peptide-loaded fibroin nanoparticles was carried out in several cells considering as breast cancer cells like MDA-MB-435, MDAMB-231, and MCF10A. This study showed significant target specificity to tumorigenic cells (Numata *et al* 2012). Tian Y *et al.* 2014*,* investigated Doxorubicin-Loaded Magnetic SFNs to overcome multidrug resistance and enhanced drug accumulation at the tumor sites and it can be prepared by using one-step potassium phosphate salting-out strategy by adding a certain amount of hydrophilic magnetic Fe_3O_4

nanoparticles (MNPs) in phosphate solution. This study demonstrated that the DMSs work well as a novel drug delivery system in cancer therapy (Tian *et al* 2014).

Kaplan *et al*. investigated doxorubicin-loaded silk fibroin had greater primary tumor response than the equivalent dose of doxorubicin intravenously administered without silk film carrier. Therapeutic impact of doxorubicin-loaded silk films assessed in mice using adenocarcinoma (a humanized orthotopic breast cancer model) (Seib and Kaplan 2012). Subia B *et al*. 2013, prepared methotrexate loaded silk fibroin–albumin blended nanoparticles by desolvation method without any surfactant having size range 140–300 nm showed better encapsulation efficiency, drug loading ability, and less toxicity, released the drug to the targeted site significantly slower than the drug without silk fibroin. The diameter of SF–Alb nanoparticles significantly smaller than the other blended particles. Here cytotoxicity study was carried out in MDA-MB-231 cell line considering as breast cancer cell (Subia and Kundu 2013). Gupta *et al*. 2011, Investigated the effect of SFCS (silk fibroin and chitosan) blend scaffolds-emodin nanoparticles on tissue defect post-tumour resection by providing local release of the therapeutic and filling of the defect site with the regenerative bio-scaffolds. They have used breast cancer cell line GILM2 and found emodin inhibited the growth of cells in a dose-dependent manner (Gupta *et al* 2011). Wu P *et al*. 2013, carried out *in vivo* and *in vitro* cytotoxicity study in two human gastric cancer cell lines BGC-823 and SGC-7901 of paclitaxel loaded SF-NPs with a diameter of 130 nm formed in an aqueous media by self-assembling of SF protein at room temperature demonstrated paclitaxol kept it's pharmacological activity when it is incorporated into SF-NPs, while SF showed no cytotoxicity to cells. This study showed superior antitumor efficacy for gastric cancer treatment by reducing tumor weights and delaying tumor growth (Wu *et al* 2013). Yu S *et al*. 2014, prepared hydrophilic anti-cancer drug loading regenerated silk fibroin (RSF) nanoparticles as a drug carrier by using a facile and clean method based on the self-assembly of silk protein. Average particle size ranging from 210-510 nm having drug loading capacity 6.8%, releasing the drug in more than 2 days which exhibits a curative effect of killing or inhibiting Hela cells (Yu *et al* 2014). Yucel T *et al*., investigated the viability of the silk reservoir rod technology for sustained drug delivery using Anastrozole as a model anticancer drug. Film spinning-end sealing method was used to prepare silk-anastrozole reservoir rods that showed desirable pharmacokinetics profile, biodegradation, and biocompatibility that may be suitable for sustained delivery of breast cancer therapeutics (Yucel *et al* 2014). J. Wang *et al*. prepared doxorubicin hydrochloride incorporated silk fibroin nanoparticles ranging from 100 to 500 nm used as a sustained release drug delivery system to treat cancer where silk fibroin was extracted from Chinese oak tasar (Antheraea pernyi) and nanoparticles were prepared

by ion-induced self-assembly. The experiment carried out in human hepatocarcinoma cells (HepG2) and the result has shown potential to cancer treatment (Wang *et al* 2015). S.K. Cheema *et al*. 2007, investigated SF-ELP (silk fibroin coated-emodin loaded liposomes) was more efficacious in suppressing the growth of Her2/neu as compared to uncoated emodin loaded liposomes (ELP) over-expressing breast cancer cells MDA-MB-453 and BT-474(Cheema *et al* 2007). Seib *et al*., investigated doxorubicin loaded SF nanoparticle (98 nm) prepared by absorption showed pH-dependent release (pH 4.5>> 6.0 > 7.4) *in* vitro and it able to overcome drug resistance mechanisms which demonstrated drug-loaded silk nanoparticle's ability to serve as a lysosomotropic delivery platform (Seib *et al* 2013).

v. SF nanoparticle for ocular targeting

Ocular drug delivery is one of the most challenging pharmaceutical tasks, because of the critical and unique environment in the eye. For instance, most of the topically applied drugs will be washed off quickly from the surfaces of the eyes by various mechanisms, such as lacrimation, tear dilution and tear turnover (Baudouin 1996). Researchers have focused on two ways to achieve more efficient ocular delivery: increasing the drug transport through the ocular barriers or extending the residence time of drug on the eye surface (Zurowska-Pryczkowska, Sznitowska and Janicki 1999). Using a mucoadhesive material is an efficient solution to improve ocular drug therapeutic efficacy. Dong Y *et al*. 2015, investigated a liposomal formulation coated by a novel adhesive excipient, silk fibroin (SF), for topical ocular drug delivery. The regenerated silk fibroins (SFs) with different dissolving time were coated onto the ibuprofen-loaded liposomes (SLs). Cellular adhesion and cytotoxicity assay of SF and SLs were tested using human corneal epithelial cells (HCEC). SLs showed sustained drug release up to 12 h with no detectable cytotoxicity (Dong *et al* 2015). Huang D et al. 2014, investigate the feasibility of silk fibroin nanoparticles (SFNs) for sustained drug delivery in trans-scleral ultrasound. Fluorescein isothiocyanate labeled bovine serum albumin was chosen as a model macromolecular protein drug and SFNs were used as nano-carrier systems suitable for ocular drug delivery. The posterior eye segment of rabbit was examined for adverse effect by slit-lamp and histology. It was found that FITC-BSA-SFNs possessed sustained release, bio-adhesive, and co-permeation characteristics. The ultrasound application significantly improved the penetration efficiency of FITC-BSA-SFNs as compared with passive delivery, meanwhile caused no damages to the ocular tissue and particles themselves. The distribution profile of SFNs revealed rapid and lasting adhesion on the

outer scleral tissues, followed by migration into the interior up to one week after treatment (Huang *et al* 2014).

Patents on fibroin nanoformulations (Table 2)

Zhang Y 2005 filed a patent on Manufacture process of nano fibroin particle. The invention discloses a method of making nano fibroin particles by silks, mixing water soluble fibroin solution with an excessive organic solvent to causing the fast beta conversion of the fibroin molecular structures so as to form crystal particles, eliminating organic solvent, dispersing and obtaining highly pure crystalloid nano fibroin particles. The particle is spherical, doses not dissolve in water, and has an average size of 30-60 nm, a beta-folded structure, a crystallinity of 18-25%, about half of that of the natural fiber, has no toxicity, harm and immune effect to human bodies, has excellent biocompatibility and can effectively stop UV radiation, makes the bacteria unable to normally grow and breed in the nano fibroin water solution, and can be widely applied to cosmetics, skin protecting articles, synthetic materials, surface modified materials as well as sustained release carrier of enzyme, polypeptide, and drugs, etc. (Zhang 2005). Zhang Y filed a patent on Silk nanogranular of immobilized enzyme, and preparation process thereof. This invention discloses a method for manufacturing silk fibroin nanoparticles fixed with enzyme from silk fibroin, which comprises the steps of: completely mixing the enzyme and regenerated silk fibroin solution, injecting into a water-soluble organic solvent under high speed stirring to obtain white crystalline silk fibroin nanoparticles fixed with the enzyme and centrifuging or filtrating to remove the organic solvent to obtain crystalline silk fibroin nanoparticles fixed with the enzyme. The average particle size of the nanoparticles is 35-125 nm, and the activity recovery is as high as 70%. The fixed enzyme has a high thermal stability and is not easy to be decomposed by proteinase, thus can largely reduce or even eliminate the immunogenicity of the enzyme. The nanoparticle fixed with the enzyme has important applications in sustained drug release, industrial enzyme reactor, food additives and cosmetics (Zhang 2006). Lian ZF filed a patent on Method for preparing fibroin nanoparticles taking polyvinyl alcohol as a stabilizer. The method comprises the following process steps of: preparing fibroin extracted from silk into 58% by weight in volume of fibroin solution, adding a polyvinyl alcohol solution into the fibroin solution to obtain a blended solution, dropwise adding the blended solution into a water-soluble organic solvent which can be used for inducing beta folding of the fibroin, and stirring continually for 16 hours to obtain a fibroin nanoparticle solution, further purifying and enriching the fibroin nanoparticle solution to obtain uniform fibroin nanoparticles; and storing the obtained fibroin nanoparticles.

According to the preparation method, fibroin nanoparticles are suitable for large scale production and are widely applied in a plurality of fields such as medicament release, patellofemoral joint biological lubricants, face lifting, beauty treatment, tissue repair and the like (Lian 2012). Jidong Z 2014 filed a patent on Method for preparing fibroin nanoparticles by virtue of activated PEG (polyethylene glycol). The method comprises the following steps: degrading a dialyzed fibroin solution to a smaller molecular weight by utilizing enzymatic dissolution, dropwise adding an activated PEG solution into the sodium tetraborate containing fibroin solution at low temperature, leaving the mixed solution to stand to obtain a fibroin nanoparticle solution, and performing centrifugal washing and drying to obtain high monodispersity fibroin nanoparticles. According to the method, the molecular weight distribution of fibroins is controlled by virtue of enzymatic degradation, and the self-assembly of the fibroins is promoted by virtue of the activated PEG, so that the obtained fibroin nanoparticles has small particle sizes, and are uniform in distribution, safe and nontoxic (Jidong 2014).

Conclusion and future scope

Many efforts have been made to improve the therapeutic efficacy of biomolecules for various biomedical applications. Nanotechnology has led to the development of nanoparticle-based drug delivery vehicles in order to overcome the side effects associated with chemotherapeutics. Silk-based nanoparticles have been developed to target lung, tumor and ocular disease for the delivery of proteins, small molecules, and anticancer drugs as described in this review. SF has many unique characteristics, including appropriate mechanical properties, versatile process ability in an aqueous environment, biocompatibility, and a controlled degradation rate that make it an excellent candidate for drug delivery applications. For this reason, SF nanoparticles have been successfully designed and are able to control the release rate of biomolecules in a sustained manner with high stability.

Nevertheless, there are limitations that need to be overcome to explore the complete potential of silk fibroin. A greater understanding of the fundamentals underlying structure–function relationship of these proteins is needed to control the properties according to the specific requirements of design criteria. When some nanoparticles are prepared from silk fibroin for advanced applications, rigorous and accurate mathematical modeling will be required to describe the systems and the mechanisms associated with their drug release. Consistent with several other natural and synthetic polymers and as described herein, silk nanoparticles support encapsulation of cells and therapeutic

Table 2: Patents on Fibroin-based Nanoformulations.

Patent Number	Title	Claim (s)	Reference (s)
CN1560136 A	Manufacture process of nano fibroin particle	1. Crystalline silk fibroin nanoparticles suspension or nanopowder was prepared by nanowire fabrication method. 2. The nanoparticle is spherical, doses not dissolve in water, and has an average size of 30-60 nm, a beta-folded structure, a crystallinity of 18-25% 3. The nanoparticle has excellent biocompatibility and can effectively stop UV radiation, makes bacteria unable to normally grow and breed in the nano fibroin water solution, and can be widely applied to cosmetics, skin protecting articles, synthetic items.	(Zhang 2005)
CN1834240 A	Silk nanogranular of immobilized enzyme, and preparation process thereof	1. An immobilized enzyme fibroin nanoparticles comprising: silk fibroin it as the core, the enzyme is embedded and fixed in the surface of the particles was prepared. 2. The average particle size of the nanoparticles is 35-125 nm, and the activity recovery is as high as 70%. 3. The fixed enzyme has a high thermal stability and is not easy to be decomposed by proteinase, thus can largely reduce or even eliminate the immunogenicity of the enzyme.	(Zhang 2006)

Continued…..

Table 3: Patents on Fibroin-based Nanoformulations (Continued….)

Patent Number	Title	Claim (s)	Reference (s)
CN102344686 A	Method for preparing fibroin nanoparticles taking polyvinyl alcohol as a stabilizer	1. The invention relates to a method for preparing fibroin nanoparticles taking polyvinyl alcohol as a stabilizer. 2. The particle formed comes under the uniform size of 100-200 nm. 3. The fibroin nanoparticles are suitable for large scale production and are widely applied in a plurality of fields such as medicament release, patellofemoral joint biological lubricants, face-lifting, beauty treatment, tissue repair etc.	(Lian 2012)
CN104231283 A	Method for preparing fibroin nanoparticles by virtue of activated PEG (polyethylene glycol)	1. The invention discloses a method for preparing fibroin nanoparticles. The molecular weight distribution of fibroins is controlled by enzymatic degradation, and the self-assembly of the fibroins is promoted by the activated PEG. 2. The method comprises degrading a dialyzed fibroin solution to a smaller molecular weight by utilizing enzymatic dissolution, dropwise adding an activated PEG solution into the sodium tetraborate containing fibroin solution at low temperature, to obtain a fibroin nanoparticle solution, followed by washing and drying. 3. The obtained fibroin nanoparticles have small particle sizes (249-681nm) and are uniform in distribution, safe and nontoxic.	(Jidong 2014)

molecules. They are porous to allow diffusion of nutrients and wastes as well as to allow intercellular communication with secreted molecules. The simplicity of adjusting release profiles by tools such as varying its concentration, applying multiple coatings or changing the β-sheet content, makes SF especially attractive. Moreover, simple surface modifications may affect drug binding and release and even allow targeted drug delivery. The option to combine different SF drug delivery systems in one construct leads to a sheer unlimited range of drug delivery devices providing distinct release kinetics.

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