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# Progress in silk and silk fiber-inspired polymeric nanomaterials for drug delivery

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The fields of drug and gene delivery have been revolutionized by the discovery and characterization of polymer-based materials. Polymeric nanomaterials have emerged as a strategy for targeted delivery because of features such as their impressive biocompatibility and improved availability. Use of naturally derived polymers in these nanomaterials is advantageous due to their biodegradability and bioresorption. Natural biopolymer-based particles composed of silk fibroins and other silk fiber-inspired proteins have been the focus of research in drug delivery systems due to their simple synthesis, tunable characteristics, and ability to respond to stimuli. Several silk and silk-inspired polymers contain a high proportion of reactive side groups, allowing for functionalization and addition of targeting moieties. In this review, we discuss the main classes of silk and silk-inspired polymers that are being used in the creation of nanomaterials. We also focus on the fabrication techniques used in generating a tunable design space of silk-based polymeric nanomaterials and detail how that translates into use for drug delivery to several distinct microenvironments.

KEYWORDS

**polymeric nanoparticles, natural biopolymers, silk, drug delivery, nanomaterials**

## Introduction

The efficacy of treatment for numerous diseases and conditions including, but not limited to, cancers, autoimmune disorders, inflammation, and infection, is limited by how therapeutic agents and drugs are delivered. Systemically administered drugs suffer from low water solubility, rapid clearance, and can cause a variety of off-target side effects (Jain, 2020). Administering drugs *via* alternative routes is an area of interest, but overcoming the body's natural barrier systems is a significant challenge. To address the limitations associated with systemic delivery, research focused on advanced drug delivery systems (DDS) has been a major area of interest (Hrkach and Langer, 2020). Nanomaterial-based DDS, such as nanoparticles, liposomes, and dendrimers, have been shown to enhance the bioavailability of various therapeutics (Blanco et al., 2015; Brannon et al., 2022). A major class of nanomaterial DDS are polymeric nanomaterials engineered to include features

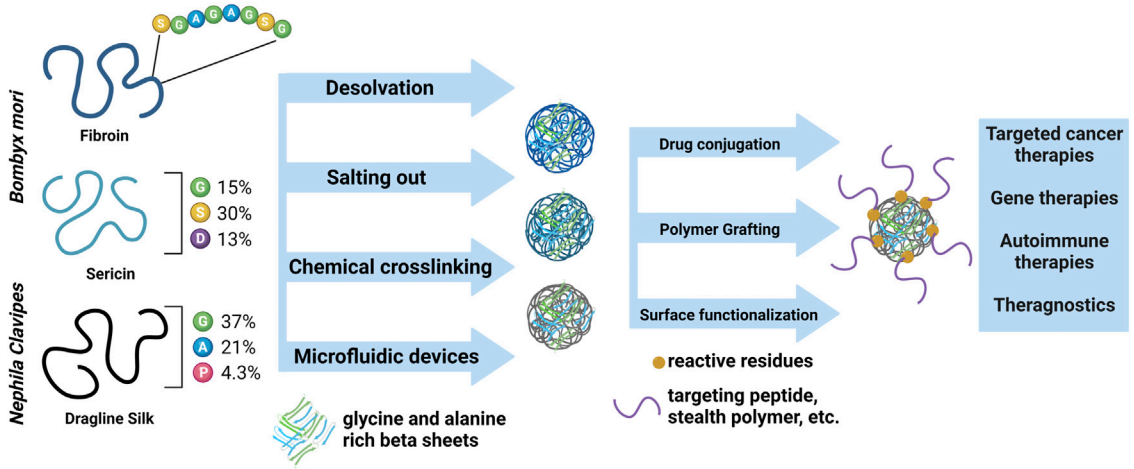


FIGURE 1
Common methods for silk biopolymer-based nanomaterial fabrication and functionalization.

such as targeting moieties (Chen et al., 2021a). Using natural biopolymers for the creation of these DDS is further advantageous due to their impressive biocompatibility and biodegradability (George et al., 2019). The main classes of natural biopolymers used for the synthesis of nanoparticle-based systems are polysaccharides and proteins.

Silk fibroins and other silk fiber-inspired proteins have been shown to be biocompatible and easily digested by immune cells and proteolytic enzymes into products that can re-enter native metabolic processes on a tunable timeline (Guo et al., 2020; Jameson et al., 2021). Apart from their biocompatibility and tunable biodegradation, silk proteins are also advantageous polymers for the engineering of DDS due to the myriad of methods to induce increases in crystalline content. This translates into numerous methods for engineering nanomaterials of various sizes, depending on the end state application, that do not require the use of harsh solvents (Pham and Tiyaboonchai, 2020). Increased crystalline content also limits the tendency for burst release behavior (Wongpinyochit et al., 2019; Pham and Tiyaboonchai, 2020). The main silk proteins that have been explored for use in a variety of nanoparticle DDS are silk fibroins from the *Bombyx mori* silkworm. *Bombyx mori* silk fibroin is the most well-characterized silk protein source and has a variety of reactive amino acids to allow for functionalization (Murphy et al., 2008; Murphy and Kaplan, 2009; Asakura et al., 2015; Heichel and Burke, 2020; Bittencourt et al., 2022). Other silk proteins, including *Bombyx mori* sericin, genetically engineered silk fibroin proteins, silk fibers from other *Antheraea* species, and bioengineered spider fibroin silks (Bittencourt et al., 2022), are being explored due to their enhanced ability for functionalization, potential for a wider range of material and mechanical properties, and increased homogeneity of the starting polymer. In this review, we discuss the primary structure of these silk proteins and relate the structure to fabrication strategies for nanomaterials (Figure 1). We also highlight progress in the *in vitro* and *in vivo* evaluations of silk-based DDS.

## Fabrication and evaluation of nanomaterial DDS based on silk proteins and silk fiber-inspired polymers

### *Bombyx mori* fibroin

*Bombyx mori* silk is composed of two main protein groups, fibroin and sericin. Two fibroin fibers are surrounded by a sericin coating. When fibroin and sericin are present together, there is decreased biocompatibility (Ode Boni et al., 2022). Sericin is water soluble, allowing for fibroin to be readily extracted (Rockwood et al., 2011). Historically, sericin has been discarded as waste, but we discuss the potential for sericin nanomaterials in a later section.

Fibroin from *Bombyx mori* is biocompatible and the focus of biomedical research for applications including nanomaterials and both local and systemic DDS. Fibroin consists of heavy (~325 kDa) and light (~26 kDa) chains linked with a disulfide bond (Zhou et al., 2001). The light chain is elastic, hydrophilic, and characterized by nonrepeating sequences of amino acids. The heavy chain comprises highly hydrophobic and crystalline motifs (GAGAGS or GAGAGX, X = V or Y) separated by amorphous regions containing polar and charged amino acids (Zhou et al., 2001). The hydrophobic motifs can form layers of

TABLE 1 Summary of current advances in silk and silk fiber-inspired nanomaterials for drug and gene delivery.

| Polymer source | Fabrication strategy | Particle size | Application | *In vitro* and/or *in vivo* models | Refs |
|---|---|---|---|---|---|
| *B. mori* fibroin | Chemical crosslinking | 50–100 nm | Molecular imprinting | *In vitro:* 3T3 cytotoxicity | Bossi et al. (2021) |
| | Nanoprecipitation | 200–220 nm | pH responsive Silk-ZIF-8 core-shell nanospheres, Doxorubicin delivery | *In vitro:* MCF-7 cytotoxicity, uptake and localization<br>*In vivo:* subcutaneous injection in BALB/c nude mice safety evaluation<br>MCF-7 tumor baring mice-therapeutic anti-tumor effect | Chen et al. (2021b) |
| | Desolvation | 175–225 nm | NAC nasal delivery | *In vitro:* TEER assays and tight junction analysis in human nasal RPMI 2650<br>*In vivo:* Sprague Dawley IVIS study | Chung et al. (2022) |
| | Desolvation | 110 nm | Celecoxib and curcumin delivery for osteoarthritis | *In vitro:* isolated articular chondrocyte inflammation model | Crivelli et al. (2019) |
| | Desolvation | 200–300 nm | cRGD functionalized silk for targeted delivery of patchouli alcohol | *In vitro:* anti-inflammation assays in RAW 264.7 macrophages, Intestinal barrier protection evaluation in Caco-2 cells<br>*In vivo:* biodistribution and therapeutic outcomes evaluation in mice | Du et al. (2022) |
| | Spray drying | ~165–260 nm | Rosmarinic acid delivery to cancer cells | *In vitro:* cellular uptake, and cytotoxicity assays | Fuster et al. (2021) |
| | Desolvation | 300–400 nm | NO delivery | *In vitro:* viability assays with 3T3 cells, antibacterial effect on viability with *E. coli* and *MRSA* | Ghalei et al. (2020) |
| | Desolvation | 100–180 nm | Silk particles as adjuvants in subunit multi epitope vaccine for UTI | *In vitro:* cytotoxicity in L929 cells<br>*In vivo:* immunization assay in BALB/c mice | Hasanzadeh et al. (2020) |
| | Desolvation | ~160–190 nm | Targeted delivery of ferulic acid by neutrophil coating of silk particles for pancreatitis | *In vivo:* IV injection in male Wistar rats. Biodistribution and therapeutic effect | Hassanzadeh et al. (2021) |
| | *ionic gelation, green synthesis<br>Chemical crosslinking<br>*processing steps not involving silk | ~8 nm,<br>~1 μm | Chitosan-gold nanoparticles coated in functionalized silk fibroin for targeted delivery of doxorubicin | *In vitro:* HeLa cell viability and live cell imaging | Horo et al. (2021) |
| | Emulsion polymerization | 38–220 nm | PEGylated fibroin nanoparticles for 5-FU delivery in treatment of colorectal cancer | *In vitro:* HT-29 cytotoxicity assays | Hudita et al. (2021) |
| | Semi-batch processing using micromixing and microfluidics-desolvation | 109–149 nm | Volumetric scale up of silk particles to enhance the potential for translation | --- | Matthew et al. (2022) |
| | Desolvation and pH induced self-assembly | 106–165 nm | Respiratory delivery of rifampicin for treatment of mycobacterial disease | *In vitro:* inflammatory response of infected RAW 264.7 macrophages | Mitra et al. (2022) |
| | Desolvation | 186 nm | Delivery of tamoxifen citrate for treatment in breast cancer | *In vitro:* cytotoxicity and uptake in MCF-10A (noncancerous), MCF-7 and MDA-MB-231 (cancerous) lines | Moin et al. (2021) |
| | Self-assembly | 120–170 nm | Delivery of gene therapies for treatments of lung cancer by chemically modified, cationized silk fibroin carriers | *In vitro:* transfection and viability analysis in A549 and WI-38 cells | Niu et al. (2021) |
| | Desolvation | 69 nm | Delivery of antioxidant and imaging agents (theragnostic) | *In vitro:* cytotoxicity in L132 and A549 cells | Passi et al. (2020) |
| | Desolvation and chemical crosslinking | 300–500 nm | Delivery of paclitaxel for colon cancer treatment | *In vitro:* cytotoxicity and uptake in Caco-2 and MCF-7 cells | Pham et al. (2020) |
| | Desolvation | 92–200 nm | Composite silk fibroin/alginate nanoparticles in PNIPAM hydrogel for pH sensitive delivery of vancomycin for treatment of infection in chronic burns | *In vitro:* biocompatibility, cell migration analysis with L929 cells<br>*In vivo:* infected burn model in male Wistar albino rats | Rezaei et al. (2020) |

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TABLE 1 (*Continued*) Summary of current advances in silk and silk fiber-inspired nanomaterials for drug and gene delivery.

| Polymer source | Fabrication strategy | Particle size | Application | *In vitro* and/or *in vivo* models | Refs |
|---|---|---|---|---|---|
| | Salting out | 107–436 nm | Magnetic silk + polyethyleneimine core shell for targeted delivery of gene therapies in treatment of breast cancer | *In vitro:* cytotoxicity and uptake analysis in MDA-MB-231 or HDF cells | Song et al. (2019) |
| | *thin film hydration<br>Desolvation<br>*processing steps not involving silk | 136–191 nm | Long-term delivery of cancer therapies (doxorubicin) | *In vitro:* cytotoxicity in L929, MDA-MB-231 and MCF-7 cells | Suyamud et al. (2021) |
| | Desolvation | 36–48 nm | Transdermal deliveries of therapies | *In vivo:* transdermal distribution study in male ICR mice | Takeuchi et al. (2019) |
| | Microfluidic assisted desolvation | 129–232 nm | Targeted delivery by G3 functionalized particles of cancer therapy ASC-J9 | *In vitro:* cytotoxicity, uptake, and tumor spheroid penetration in HCT-116 and DF cells | Tomeh et al. (2022) |
| | Co-flow microfluidic device assisted desolvation | ~6 μm | Doxorubicin delivery to tumor microenvironment | *In vitro:* cytotoxicity, inflammation response, uptake in KELLY and THP-1 cells | Vargas Montoya et al. (2020) |
| | Water/oil emulsion | 20–140 μm | Injectable microspheres analysis for future delivery in treatment of osteoarthritis | *In vitro:* cytocompatibility analysis with rat bone MSCs<br>*In vivo:* subcutaneous injections in Sprague Dawley rats for biocompatibility analysis, ACL transection model in Sprague Dawley rats | Zhang et al. (2019) |
| | Concentration followed by incubation to induce self-assembly | 100 nm-1 μm | Delivery of cancer therapies (doxorubicin) | *In vitro:* uptake, retention, cytotoxicity analysis in HUVEC and MCF-7 cells. Tumor spheroid penetration (MCF-7) | Xiao et al. (2022) |
| | Desolvation | 179 nm | Delivery of biotherapeutics to the retina | *In vitro:* cytotoxicity, uptake, retention analysis in ARPE-19 cells<br>*In vivo:* biodistribution and safety of intravitreal injection in white New Zealand rabbits | Yang et al. (2019) |
| | Desolvation and freezing induced self-assembly | 330–379 nm | Delivery of gene therapies | *In vitro:* cytotoxicity, uptake, immunostimulation in RAW 264.7 | Zhang et al. (2019) |
| | Desolvation | 53–73 nm | $MnO_2$ capped silk fibroin particles for photodynamic therapy for cancer | *In vitro:* viability in L929 and HUVEC cells. Cytotoxicity and uptake in 4T1 cells<br>*In vivo:* evaluation of biodistribution, pharmacokinetics, and antitumor effects in a 3T1 tumor model in BALB/c mice | Zhang et al. (2021) |
| | Desolvation | 120 nm | Indocyanine green loaded silk particles for photo-thermal treatment of glioma | *In vitro:* cytotoxicity and uptake in RAW 264.7 and C6 cells. Photo-thermal effect with C6 cells<br>*In vivo:* biodistribution, pharmacokinetics, and antitumor effects in C6 tumor model in BALB/c mice | ZhuGe et al. (2019) |
| *B. mori* sericin | Self-assembly in water | 35–370 nm | Sericin-PLA are chemically conjugated to form an amphiphilic material for doxorubicin delivery | *In vitro:* cytotoxicity and uptake in HepG2 cells | Boonpavanitchakul et al. (2020) |
| | Self-assembly in DMSO with Pluronic F-127 | 137–201 nm | Slow release of antioxidant polyphenols for support of regeneration by MSCs | *In vitro:* cytocompatibility and proliferation assays in human adipose derived MSCs | Orlandi et al. (2020) |
| | Chemical crosslinking followed by desolvation | 225–248 nm | Evaluating crocetin as novel crosslinker for bioactive sericin particles for nasal delivery and limiting oxidative stress | *In vitro:* metabolic activity assay in Caco-2 cells | Perteghella et al. (2021) |

(Continued on following page)

TABLE 1 (*Continued*) Summary of current advances in silk and silk fiber-inspired nanomaterials for drug and gene delivery.

| Polymer source | Fabrication strategy | Particle size | Application | *In vitro* and/or *in vivo* models | Refs |
|---|---|---|---|---|---|
| | Nanoprecipitation | 100–300 nm | Doxorubicin delivery in treatment of breast cancer | *In vitro:* cytotoxicity, cytoskeletal changes, and comet assays in MCF-7 cells | Radu et al. (2021) |
| | Desolvation | 123–147 nm | pH responsive, targeted delivery of lactoferrin for treatment of ulcerative colitis | *In vitro:* cytotoxicity, cellular uptake, and anti-inflammatory activity in RAW 264.7 cells<br>*In vivo:* ulcerative colitis model in BALB/c mice | Xu et al. (2022) |
| Non-*B. mori* native silks | Desolvation and radiolysis | 180–250 nm | Characterization of secondary structure of *Antheraea assamensis* fibroin nanoparticles for applications in controlled release | --- | Asapur et al. (2022) |
| | Desolvation | 185 nm | Release of doxorubicin from *Antheraea assamensis* fibroin nanoparticles for cancer treatment | *In vitro:* biocompatibility in L929 cells. Cytotoxicity in MDA-MB-231 cells | Baruah et al. (2020) |
| | Desolvation | 400–800 nm | Stimuli responsive, PEG-*Antheraea pernyi* silk particles for gene therapy in cancer | *In vitro:* transfection and cytotoxicity assays in HT1080 and HEK293 cells | Liu et al. (2021) |
| | Self-assembly | 109–155 nm | Chitosan and *Antheraea mylitta* silk-coated silver nanoparticles for transdermal cancer therapy delivery | *In vitro:* cytotoxicity, comet, morphology assays in A431 and HaCaT cells<br>*In vivo:* immune response in BALB/c mice | Nayak et al. (2021) |
| Recombinant silks | Salting out | ~400 nm | Targeted delivery of doxorubicin for cancer in particle inspired by *Nephila clavipes* spider | *In vivo:* biodistribution and safety in BALB/cAnNCrl mice | Deptuch et al. (2021) |
| | Salting out | ~400 nm | Targeted delivery of doxorubicin for cancer in particle inspired by *Nephila clavipes* spider | *In vitro:* cytotoxicity, and uptake in D2F2E2/LUC and D2F2/LUC cells<br>*In vivo*-models for orthotopic and metastatic breast cancer in BALB/cAnNCrl mice | Florczak et al. (2020) |
| | Salting out | ~1 μm | Stimuli responsive *Araneus diadematus* inspired particle for cancer therapy delivery | *In vitro:* cytotoxicity and uptake in HeLa cells | Herold et al. (2020) |
| | Nanoprecipitation | 184 nm | Stimuli responsive *Nephila clavipes* inspired particles for antibiotic delivery | *In vitro:* infection responsive activity *in Staphylococcus* aureus and *Staphylococcus epidermidis*<br>*In vivo:* septic arthritis model in Sprague Dawley rats | Mulinti et al. (2021) |
| | Salting out | ~1 μm | *Araneus diadematus* inspired particles for directing corona formation, applications in systemic delivery | *Ex vivo:* incubation in human whole blood for corona analysis | Weiss et al. (2020) |

beta-sheet structures, thereby altering the crystallinity and mechanical properties of fibroin-based materials. The level of crystallinity varies with the material fabrication methods and can be further increased using post-processing techniques such as water annealing (Hu et al., 2011; Rockwood et al., 2011; Jameson et al., 2021). Fibroin nanoparticles have also been shown to have stimuli-responsive behavior, with increased degradation and drug release occurring as a function of stimuli such as pH and the presence of reactive oxygen species, making them advantageous for delivery of therapies like chemotherapeutics (Seib et al., 2013; Rezaei et al., 2020; Chen et al., 2021b).

To form nanomaterials with silk fibroin, researchers have developed an array of physical and chemical methods to induce the hydrophobic collapse of the hydrophobic motifs and encourage the formation of water insoluble nanoparticles (Murphy et al., 2008; Murphy and Kaplan, 2009; Wenk et al., 2010; Heichel and Burke, 2020; Hong et al., 2020). Recent studies have utilized techniques such as spray drying (Fuster et al., 2021),

organic solvent desolvation (Hasanzadeh et al., 2020; Rezaei et al., 2020; Zahedi et al., 2021), salting out (Song et al., 2019), and microfluidic devices (Shimanovich et al., 2017; Matthew et al., 2020; Vargas Montoya et al., 2020; Matthew et al., 2022; Tomeh et al., 2022) (Table 1). Control of particle size and material properties to enhance cell material interaction (Xiao et al., 2022) and to optimize non-systemic delivery routes has been established (Takeuchi et al., 2019; Yang et al., 2019; Chung et al., 2022; Mitra et al., 2022).

Recently, researchers have combined functionalization techniques with the use of composite materials to generate the next generation of silk-based nanomaterials that can function as targeted delivery systems (Song et al., 2019; Totten et al., 2019; Pham et al., 2020; Bossi et al., 2021; Hassanzadeh et al., 2021; Horo et al., 2021; Zahedi et al., 2021; Du et al., 2022) and as systems for both therapy and diagnostics (ZhuGe et al., 2019; Passi et al., 2020; Zhang et al., 2021). Pham et al. (2020) developed a fibroin nanoparticle system for delivery of therapeutics in the treatment of colon cancer. They encapsulated paclitaxel in fibroin particles (300–500 nm) formed by organic solvent desolvation and EDC crosslinking. They found their formulations were effective in colon and breast cell cancer lines, but interestingly observed that when the formulation was used in colon cancer lines, cytotoxic efficiency increased 10-fold relative to free drug (Pham et al., 2020). Du et al. (2022) also explored targeting sites in the colon for ulcerative colitis. They formed nanoparticles by desolvation and then surface functionalized them with cRGD. Safety and efficacy were evaluated *in vitro* and *in vivo* and it was found that the cRGD functionalized particles specifically interacted with integrin αv inflammation sites in the colon and contributed to an improved therapeutic outcome (Du et al., 2022). Horo et al. (2021) developed a composite material for targeted delivery of antitumor agents. They developed chitosan-stabilized gold nanoparticles (~8 nm) loaded with doxorubicin and coated with fibroin conjugated with folic acid and fluorescein to promote the targeting of tumor cells. The silk coating contributed to an improved drug release profile and increased uptake and cytotoxicity in HeLa cultures. They also modified this formulation into larger particles (~1,000 μm) using ionic gelation to address the potential for oral delivery (Horo et al., 2021). Song et al. (2019) also explored anti-cancer composite silk nanomaterials for targeted delivery for gene therapies. They utilized a salting out technique to fabricate magnetic silk + polyethyleneimine core shell nanoparticles with tunable size (~100–500 nm). They demonstrated enhanced delivery of therapeutic antisense oligodeoxynucleotides by use of magnetofection in MDA-MB-231 breast cancer cells (Song et al., 2019). Silk-based nanomaterial DDS continue to be made of primarily *Bombyx mori* fibroin polymers, but recently researchers have also begun investigating other silk fiber biopolymers as well as alternative sources for silk fibroin biopolymers that have additional unique properties for use in biomedical applications.

## *Bombyx mori* sericin

As mentioned previously, sericin proteins from *Bombyx mori* cocoons have traditionally been discarded as a waste product. However, recent work has highlighted how, when purified, sericin proteins are non-cytotoxic, have a high potential for reactive modification, and can facilitate cell-material interaction. A major limitation of sericin proteins is that they are highly water soluble, meaning the majority of nanomaterials made with sericin must be composite materials. Sericins from *Bombyx mori* are not as well characterized as their fibroin counterpart, but it has been shown that they are globular proteins in nature ranging from 20 to 310 kDa and mainly consist of reactive residues like serine and aspartic acid. This means sericin materials often have increased presence of random coil structures; however, crystallinity can still be induced in the material synthesis process by techniques such as desolvation. Recent studies utilizing sericin-based DDS have focused on applications including nasal delivery (Perteghella et al., 2021), pH responsive materials (Xu et al., 2022), antibacterial activity (Baker et al., 2020; Al Masud et al., 2021), cell therapy (Orlandi et al., 2020), and cancer therapies (Boonpavanitchakul et al., 2020; Baker et al., 2021; Radu et al., 2021) (Table 1).

To assemble sericin-based nanoparticles, researchers often have taken a two-step approach. First, taking advantage of the reactiveness of sericin, they chemically crosslink or modify the polymer. This is typically followed by a self-assembly or desolvation step. Boonpavanitchakul et al. (2020) conjugated a hydrophobic polymer, PLA, to sericin, forming an amphiphilic material. They were then able to self-assemble the PLA-silk sericin polymers into nanomaterials of tunable size (~36–370 nm) depending on the pH and inclusion of additional crosslinkers, all taking place in aqueous conditions. They further demonstrated the potential of this system as a cancer therapy by showing effective loading of doxorubicin, enhanced release under acidic conditions, and effectiveness *in vitro* liver cancer cell models (Boonpavanitchakul et al., 2020). Perteghella et al. (2021) stabilized sericin by crosslinking with crocetin and formed ~225 nm nanoparticles by desolvation in acetone. Perteghella et al. (2021) and colleagues are working toward optimizing these particles for nose-to-brain delivery and demonstrated cytocompatibility and protection from oxidative stress in Caco-2 epithelial cells. Xu et al. (2022) took a novel approach and optimized a transgenic silkworm to produce silk that is sericin-rich and has recombinant lactoferrin expressed in the sericin layer for treatment of ulcerative colitis. They were then able to synthesize ~125 nm negatively charged sericin-lactoferrin nanospheres by acetone desolvation. The effectiveness of the system was evaluated by oral delivery in an ulcerative colitis mouse model. They demonstrated improved uptake efficiency (attributed to charge interactions) and inhibition of the NF-κβ inflammatory pathway associated with acute colitis (Xu et al.,

2022). Overall, the recent progress in sericin DDS highlight the advantages of the increased bioactivity and reactivity of the protein. Researchers have also begun to evaluate the potential of silk from different species for this same purpose.

## Non-*Bombyx mori* native silk fibers

While most silk-based healthcare materials have traditionally been reverse engineered from *Bombyx mori* silk fibers, most insects in the Lepidopteran order produce silk fibers for a variety of applications (cocoon/web formation, egg coating, etc.,). Recent studies have found that some of these fibers expand the design space for silk-based materials by having more reactive residues and different mechanical properties. Recently, silk fibroin from *Antheraea assamensis* (Baruah et al., 2020; Asapur et al., 2022), *Antheraea pernyi* (Liu et al., 2021), and sericin from *Antheraea mylitta* (Nayak et al., 2021) have all been used in the development of nanoparticle DDS (Table 1).

Asapur et al. (2022) characterized the secondary structure of nanoparticles synthesized from *Antheraea assamensis* fibroin by desolvation and radiolysis methods. The fibroin from *Antheraea assamensis* is known for its ability to absorb high levels of UV radiation and its impressive tensile strength (Goswami et al., 2020). Like *Bombyx mori* fibroin, alanine-rich repeating motifs give this biopolymer the potential to form nanomaterials with high levels of β sheet crystallinity (Gupta et al., 2015). Asapur et al. (2022) found that the radiolysis method led to the formation of smaller ~180 nm particles compared to ~250 nm particles made with desolvation. Both particle types also had >30% crystalline content, showing potential for controlled release. Baruah et al. (2020) evaluated the release of doxorubicin from *Antheraea assamensis* fibroin particles made by desolvation and found efficient drug loading, and that the DDS increased the effectiveness relative to free doxorubicin of the cytotoxic effect on MDA-MB-231 cancer cells.

Liu et al. (2021) took advantage of the abundant reactive sites present in silk fibroin from *Antheraea pernyi* in the creation of their stimuli responsive DDS. They prepared particles from cationized *Antheraea pernyi* fibroin for gene delivery to cancer cells by desolvation in DMSO. Using the reactive capabilities of this fibroin, they grafted on an MMP-2 cleavable PEG to the particles surface. This allowed for limiting opsonization without sacrificing transfection when MMP-2 (highly expressed in the tumor microenvironment) cleaves the PEG *in vitro*. They demonstrated decreased protein adhesion and successful transfection to HEK293 cells (Liu et al., 2021). In addition to leveraging the biodiversity of silk fiber producing species to design novel polymeric nanomaterials for DDS, researchers have also recently been producing recombinant proteins inspired by silk fibers to create targeted DDS.

## Silk fiber-inspired recombinant polymers

As an alternative to use of purified silk fibroin proteins, researchers have also designed recombinant silk proteins inspired by nature to make polymeric nanomaterials for drug and therapeutic delivery. One approach is to use bacteria, such as *E. coli*, to express silk-like peptides either alone or in combination with other useful protein sequences (Huang et al., 2015; Bowen et al., 2018; Yu et al., 2022). Another approach utilizes whole organism genetic modification to express engineered silk fibroins or other related therapeutic agents (e.g., sericins) (Xu et al., 2022; Tamura et al., 2000; Tomita et al., 2003; Xu and O'Brochta, 2015; Saviane et al., 2018; Baci et al., 2021a; Baci et al., 2021b; Zhong et al., 2011).

Much of the focus of genetic engineering of silk proteins is based on the silk fibroins produced by spiders. This is because, unlike Lepidopteran species, spiders' cannibalistic and territorial tendencies make it so spiders cannot be farmed, meaning their fibers cannot be naturally sourced for biomedical research. Researchers have taken inspiration from the silk of *Nephila clavipes* and *Araneus diadematus* to design recombinant spider proteins. Silk produced from spiders (spidroin proteins) are ~300 kDa and composed of a highly repetitive core, mainly consisting of alanine, glycine, and proline residues, flanked by small, nonrepetitive terminal domains, which are highly conserved across spider species (Babb et al., 2017). The development, expression, and purification of spider-inspired fibroin proteins (spidroin proteins) has been recently reviewed (Kiseleva et al., 2020; Bittencourt et al., 2022). For the purposes of this review, we focus on the nanoparticle fabrication techniques recently used in the progress toward targeted DDS, and recent publications are summarized in Table 1.

The Scheibel group designed nanoparticle systems based on *Araneus diadematus* with the goal of directing corona formation and stimuli responsive release (Herold et al., 2020; Weiss et al., 2020). They recombinantly produced polymers with either a net negative or net positive charge and used the salting out technique to form nanoparticles. They then performed *ex vivo* analysis of corona formation in whole blood (Weiss et al., 2020). It was observed that the negatively charged nanospheres primarily attracted complement proteins and immunoglobulins, while positively charged particles primarily had fibrinogen-based proteins adsorbed on their surface (Weiss et al., 2020). In a separate study, researchers utilized a polyanionic form of the silk from the aforementioned study and covalently attached a cytostatic drug to the particle surface and explored the response to pH and redox state (Herold et al., 2020). Ellman's solution (a model to simulate sulfhydryl group containing drugs) was bound to cystine residues in the spider fibroin-inspired silk particle, which was then further modified by including a hydrazone linker. This system led to enhanced drug release under acidic conditions and oxidative stress in HeLa cultures (Herold et al., 2020). Researchers have also explored recombinantly producing proteins and peptides

inspired by the spidroin proteins of other spider species (Ramezaniaghdam et al., 2022).

The Dam-Kozlowska group has been building toward a cancer therapeutic DDS based on a recombinant silk fiber inspired by *Nephila clavipes* (Florczak et al., 2014; Florczak et al., 2020; Deptuch et al., 2021). They were able to produce a polymer based on the MaSp1 sequence from *Nephila clavipes* and include a Her2 binding peptide. Particles were produced by salting out and loaded with doxorubicin. They demonstrated enhanced binding to Her2-overexpressing SKOV3 cells and showed the particles themselves were noncytotoxic and could deliver doxorubicin to kill cancer cells (Florczak et al., 2014). They recently tested this system *in vivo* and demonstrated safety (Deptuch et al., 2021) and targeted delivery in breast cancer models (Florczak et al., 2020). The development of DDS based on silk nanomaterials continues to grow and researchers are moving toward potential for clinical translation.

## Future perspectives and conclusion

The wide range of material properties available from the reconstitution of silk fibers makes them an interesting class of materials for future translational research. Specifically, silk fibroins from *Bombyx mori* are able to generate a wide array of drug delivery systems (DDS) given the range of fabrication techniques, modification methods, and utility in composite materials. Furthermore, spider silk-inspired and sericin biopolymers represent additional classes of materials for DDS. Each class of materials offers its own unique features, such as biodegradation, controllable immunogenicity, and favorable mechanical properties.

While many systems have been proposed, grand challenges still exist in the application of these materials to clinical settings. As with any natural material or recombinantly produced protein, ensuring good manufacturing practices as well as methods to remove contaminating molecules or organisms, such and endotoxins or mycotoxins, is critical. Sourcing these natural materials can also be a challenge, as *Bombyx mori* cultivation and farming is subject to our changing climate and regional differences in farming practices (Gani and Ghosh, 2018; Shilpa et al., 2021), which can lead to variability in cocoons and subsequent silk properties. Recent advancements in genetic engineering technologies for the *Bombyx mori* silkworm suggest that improvements or modifications to protein structures within these systems is possible (Tamura et al., 2000; Xu and O'Brochta, 2015; Saviane et al., 2018; Baci et al., 2021a; Long et al., 2020), thus providing an avenue for addressing current challenges in protein structure highlighted throughout this review. However, any change is not without added complication or risk, as modification to silk fibroin protein sequences can pose new challenges in protein expression, spinning, or purification. Additionally, chemical modifications to existing natural materials can also alter chemistries for improvements in affinity binding and drug loading (Sato et al., 2012; Heichel and Burke, 2020). Continued efforts to further open the design space to include silk fibers or silk fiber-based proteins from other species as well as recombinantly produced polymers and peptides is another strategy for overcoming current challenges. These efforts demonstrate the enormous future potential for this class of materials and their scale-up, use in advanced manufacturing, generation of novel polymer solutions, and development of treatments for a myriad of diseases. While this review was limited to nanomaterial-based DDS, we expect that the use of novel silk proteins for the formation of materials such as scaffolds, films, foams, and hydrogels is an area of interest for local DDS, regenerative medicine, and tissue engineering. Similarly, clinical trials using materials from or synthesized to replicate components silk fibers are on-going, with a large focus on inert filler materials for treatment of conditions like vocal cord paralysis (National Library of Medicine (U.S.), 2019). These advances toward clinical application are positive first steps for the continued investigation and development of all-natural silk-inspired nanomaterials for drug delivery systems.

## Author contributions

MP: Writing-original draft, review and editing, visualization, conceptualization LE: Visualization, writing-review and editing ND: Visualization, writing-review and editing JA: Writing-review and editing AC: Writing-review and editing IK: Writing-review and editing WS: Writing-review and editing, conceptualization, supervision. All authors have read and agreed to the final version of the manuscript.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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