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Silk-elastin-like protein biomaterials for the controlled delivery of therapeutics

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Abstract

Introduction—Genetically engineered biomaterials are useful for controlled delivery owing to their rational design, tunable structure-function, biocompatibility, degradability and target specificity. Silk-elastin-like proteins (SELPs), a family of genetically engineered recombinant protein polymers, possess these properties. Additionally, given the benefits of combining semicrystalline silk-blocks and elastomeric elastin-blocks, SELPs possess multi-stimuli responsive properties and tenability, thereby, becoming promising candidates for targeted cancer therapeutics delivery and controlled gene release.

Areas covered—An overview of SELP biomaterials for drug delivery and gene release is provided. Biosynthetic strategies used for SELP production, fundamental physicochemical properties, and self-assembly mechanisms are discussed. The review focuses on sequence-structure-function relationships, stimuli responsive features, and current and potential drug delivery applications.

Expert opinion—The tunable material properties allow SELPs to be pursued as promising biomaterials for nano-carriers and injectable drug release systems. Current applications of SELPs have focused on thermally-triggered biomaterial formats for the delivery of therapeutics, based on local hyperthermia in tumors or infections. Other prominent controlled release applications of SELPs as injectable hydrogels for gene release have also been pursued. Further biomedical applications that utilize other stimuli to trigger the reversible material responses of SELPs for targeted delivery, including pH, ionic strength, redox, enzymatic stimuli and electric field, are in progress. Exploiting these additional stimuli responsive features will provide a broader range of functional biomaterials for controlled therapeutics release and tissue regeneration.

Keywords

drug delivery; gene delivery; hydrogel; nanoparticle; protein; stimuli responsive; silk-elastin; self-assembly

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1. Introduction

The delivery of therapeutics to solid tumors remains a critical problem in the treatment of cancer. Major challenges with conventional cancer therapy are poor drug efficacy and extensive toxicity to non-cancerous tissues. In particular, achieving effective drug concentration within a tumor is impeded by the low solubility of hydrophobic chemotherapeutics and transport barriers caused by the complex physiology of tumors [1]. Additionally, classical chemotherapeutics nonspecifically attack all actively dividing cells, leading to systemic toxicity and undesirable side effects. In an attempt to overcome these problems, controlled drug delivery systems using soluble macromolecular carriers have been developed and studied during the last two decades, with a focus on the design and application of ‘smart’ delivery systems that respond to the presence of specific stimuli or that mimic physiological processes [2–23]. Among the different stimuli-sensitive delivery systems being studied, genetically engineered silk-elastin-like proteins (SELPs) offer potential over alternatives due to the versatility in design, tunability of physical properties, and biocompatibility [24, 25].

SELPs are a family of genetically engineered protein block copolymers, consisting of tandemly repeated units of silk-like (GAGAGS), and elastin-like (GXGVP) peptide blocks, where X in the elastin block can be any amino acid, except proline that affects the coacervation process of elastin [26, 27]. The silk-like block, adopted as the representative sequence of the *Bombyx mori* silk heavy chain, tends to self-assemble into insoluble tightly packed secondary structures, beta sheets, to provide thermal and chemical stability, mechanical tunability and physical crosslinking sites for the SELPs polymeric system [25, 28, 29]. The elastin-like block undergoes a reversible structural transition upon exposure to the specific environmental stimulus, to endow SELPs with dynamic function [30–35]. The biological and physicochemical properties of SELPs can be specifically tuned by varying the silk-elastin ratio, the X residue in the elastin sequence and the molecular weight [25, 35] to incorporate a variety of functionalities for drug delivery, including self-assembly [25, 36–38], stimuli-sensitivity [25, 35], biorecognition and biodegradation [39, 40].

Recombinant DNA technology is usually used to generate SELPs [23, 35, 41–43] and other types of recombinant proteins [44–46]. This bioengineering approach has enabled systematic studies to correlate sequence with molecular architecture and functionality [47–52]. This approach also facilitates the integration of molecular dynamics simulations with protein design [50]. By utilizing recombinant DNA technology, the sequence, assembly, chemistry and thus material functions can be tailored and controlled, and thereby, new materials that do not exist in nature can be designed *de novo* based on sequence-structure-function relationships for specific biomedical material needs. In particular, SELPs respond to specific environmental stimuli, such as elevated temperature or reduced pH, based on the hydrophobicity of the X residue in the elastin blocks or domains [32]. By genetically modifying the elastin sequence, SELPs can be rationally designed and synthesized to target specific microenvironments for controlled release. As a bioengineering approach towards predictive stimuli responsive biomaterial design, recombinant DNA technology enables the construction of new tailor-made polymeric biomaterials with improved properties, such as stimuli-sensitivity in physiological conditions and rapid gelation for injectable hydrogels,

and thereby, this approach is key to generating new multi-stimuli responsive nanomaterials and artificial extracellular matrices for controlled release and tissue regeneration. Another advantage of recombinant proteins over synthetic polymers is their monodispersity, which improves batch-to-batch reproducibility for the pharmaceutical industry.

Prior to the studies of SELPs, thermoresponsive synthetic polymers, such as poly(N-isopropylacrylamide) (PNIPAAm) [12, 15], and several other classes of recombinant proteins, including elastin-like proteins (ELPs) [11, 53, 54], silk-like proteins (SLPs) [44–46, 55] and collagen-like proteins (CLPs) [56–59], were explored and found to have interesting self-assembling or stimuli responsive properties. PNIPAAm exhibits a lower critical solution temperature (LCST) at about 32°C in water, depending on its concentration and molecular weight. However, the main limitation of PNIPAAm is its non-biodegradability [15, 60, 61]. In contrast, recombinant proteins are biodegradable. Additionally, ELPs respond to temperature and pH via inverse temperature transitions and SLPs form beta sheets and self-assemble into micelles or vesicles. CLPs form triple helices and self-assemble into fibers. SELPs respond to environmental stimuli, such as temperature, pH, ionic strength, redox, enzymatic stimuli and electric fields, and they also form hydrogels at physiological conditions. Therefore, a interest has emerged in the use of recombinant proteins, especially SELPs, for controlled drug and gene delivery, tissue engineering and other biomedical applications. SELPs can be post-processed into various material formats, such as nanoparticles, films, thin coatings, hydrogels and scaffolds with a range of tunable properties [23, 36–39, 62–70], providing a suitable starting point for new protein-based functional materials. These SELP biomaterials have been utilized in nano-devices, biosensors, bioseparations, tissue engineering, [13, 65, 71] and most prominently, targeted drug delivery for small molecule drugs, proteins and genes [72–75].

In this review, different classes of genetically engineered SELPs and their physicochemical properties will be discussed. Various biosynthetic strategies that have been used to produce these polymers and current biomedical applications of these biomaterials will be reviewed, with emphasis on controlled release. Prior reviews of SELPs have been published [22, 53, 54, 72, 74, 76–86] to provide suitable background knowledge. The present review focuses more on the sequence-structure-function relationships of SELPs, their stimuli responsive features, and the current and potential drug delivery applications.

2. Biosynthesis of silk-elastin-like proteins

Genetically engineered proteins provide a high degree of molecular definition for correlating structure with function in controlled release [49]. In contrast to conventional synthetic polymers, the sequence and molecular weight of genetically engineered proteins can be strictly controlled by genetic templates, leading to monodisperse, precisely defined biopolymers. Moreover, the properties and function of genetically engineered proteins can be modified and optimized by appropriate mutations of the genetic template based on sequence-structure-function relationships. Therefore, several classes of functionalized genetically engineered protein polymers have been used for controlled drug delivery, including but are not limited to elastin-like [11, 20, 87], silk-elastin-like [23, 73, 74, 88, 89] and spider silk-like proteins [90, 91].

Current strategies for the genetic design and biological production of genetically engineered proteins using recombinant DNA technology have been developed and exploited for use in drug and gene delivery since the late 1980s [25, 44, 74, 77, 92–97]. The process can be summarized in three steps: (1) monomer design and plasmid construction, (2) expression, and (3) purification. Figure 1 depicts the general biosynthesis scheme. To produce SELPs, the monomers (DNA encoding the characteristic silk and elastin sequence information) are synthesized using solid-phase oligonucleotide synthesis [25]. Multimerization of these monomers is achieved through ligation, and a mixture of multimer genes with different lengths is usually generated during this concatemerization process. The mixture of the multimer genes is then ligated into expression vectors, and transformed into *E. coli* for plasmid screening. Once the DNA sequences and sizes are confirmed, the plasmid is re-transformed into a bacterial host for expression. Modified pET 19b [25, 35] and pET 25(+) [41, 43] vectors that contain an inducible T7 promoter and a purification tag are the most commonly used plasmids for SELP expression. The inducible T7 promoter allows the host bacteria to grow without expressing the cloned gene until the cells reach an appropriate cell density. The purification tag enables the use of metal chelating affinity chromatography to separate expressed protein from culture medium, dead cell debris and bacterial proteins. Currently, SELPs are mainly produced by the pET - *E. coli* BL 21 system. To achieve an optimal yield, the induction time is critical for this system [41]. In contrast to *E. coli* induction with isopropyl β -D-1-thiogalactopyranoside (IPTG) in mid log phase, optimal production of SELPs from *E. coli* BL21 was obtained by induction at the beginning of the stationary phase [41]. Yields of 25–500 mg/L by shake flask culture [25, 41, 42, 72] and 4.3g/L [43] by bioreactor cultivation were reported for purified SELPs produced in *E. coli*. However, one of the primary limitations to the successful commercialization of SELPs is still associated with the production of protein on a commercially viable level. The purification methods used for SELPs include Ni-NTA affinity chromatography [6], inverse temperature cycling [14, 16, 77, 78] and ammonium sulfate precipitation [22, 23, 25]. Purification of SELPs is conventionally carried out by Ni-NTA affinity chromatography, which utilizes histidine tag affinity to nickel above pH 6 to separate the expressed his-tag fusion proteins. This approach involves multiple binding, washing and elution steps, thus it is relatively cumbersome as well as costly. In contrast, the use of non-chromatographic approaches, such as the inverse temperature cycling and the ammonium sulfate precipitation methods, can allow for a more economical, simplified and higher throughput purification process. The inverse temperature cycling approach utilizes solubility changes of the elastin component below and above the inverse transition temperature to selectively force SELPs out of solution. Ammonium sulfate precipitation utilizes solubility changes of *E. coli* proteins and SELPs at various pHs to precipitate out *E. coli* proteins using pH 3 buffer and then SELPs using ammonium sulfate. Compared to affinity chromatography, these non-chromatographic methods avoid the use of expensive resin matrices, reduce the time and effort for protein purification, and facilitate the scale up production of SELPs to an industrial level [9].

Aside from traditional biosynthesis procedures described above, high-throughput recombinant protein synthesis and screening allow for rapid progression toward a functional material by exploiting parallel processing and appropriate screening of a large family of

starting designs simultaneously. This combinatorial approach provides a new avenue for the discovery of new SELPs starting from function rather than chemistry to assure a more rapid and efficient outcome [35].

3. Sequence-structure-function relationships of silk-elastin-like proteins

SELPs possess tunable environmental responsive properties due to the physicochemical properties of the semi-crystalline silk-like blocks and the stimuli responsive elastin-like blocks. The silk-like block (GAGAGS) in SELPs is designed to mimic the crystalline domain of *Bombyx mori* silk fibroin protein. Fourier transform infrared spectroscopy spectra [68] and circular dichroism spectra [25, 68] confirmed that insoluble tightly packed beta sheet structures were formed in silk-like blocks in SELPs. The silk units in SELPs impart the mechanical stability by forming beta sheet physical crosslinks [49, 64] and facilitate the self-assembly of SELPs into micellar-like nanoparticles by phase separation [25]. The elastin-like block (GXGVP) in SELPs is adopted from mammalian tropoelastin [98, 99]. It undergoes a reversible structural transition upon exposure to a specific environmental stimulus, such as temperature, pH, ionic strength, redox, enzymatic stimuli or electric fields, to endow SELPs with dynamic functions [34, 35, 100–102]. Turbidity tests using UV-Vis spectroscopy [25, 35] and differential scanning calorimetry [25] confirmed the reversible order-disorder transition in elastin-like blocks in SELPs. Periodic insertion of the elastin-like sequence in SELPs is primarily thought to impart viscoelastic properties and to increase solubility, while decreasing crystallinity [77, 88].

The stimuli responsive features of SELPs are governed by both the silk and elastin sequences; in particular by the silk-elastin ratio, the X residue in the elastin-like blocks, and the overall protein chain length [25, 32, 35]. The silk-elastin ratio in SELPs can be used to tune the thermal responsive properties [25]; an increased silk-elastin ratio led to a higher inverse transition temperature. The hydrophobicity of the X residue in the elastin-like block affected the stimuli responsive properties of SELPs [35]; by changing the X residue in the elastin block, SELPs respond to various stimuli including temperature, pH, ionic strength, redox, enzymatic stimuli and electric fields (Table 1). The inverse transition temperature was inversely correlated with molecular weight of the polymers [24]. The variations of SELP stimuli responsive properties as a function of sequence demonstrates the potential of using bioengineering approaches for the rational design of SELPs for controlled drug delivery and release under specify physiological conditions.

4. Silk-elastin-like protein nanoparticles for chemotherapeutics delivery

Early clinical results suggest that nanoparticle therapeutics can show enhanced efficacy, while simultaneously reducing side effects, owing to properties such as more targeted localization in tumors and active cellular uptake [105]. Enhanced permeability of the tumor vasculature allows nanoparticles to enter the tumor interstitial space, whereas the suppressed lymphatic filtration in neoplastic tissues allows additional retention of the colloidal particles in the tumor interstitium [2, 105, 106]. This phenomenon is termed ‘enhanced permeability and retention effect’ (EPR), which results in intratumoral nanotherapeutics accumulation. Nanocarriers can also alter the pharmacokinetic properties of the drug molecules and protect

the drug from premature inactivation during transport. The longer circulation time of drugs protected by nanoparticles compared with that of the free drugs alone improves tumor uptake of chemotherapeutics [107, 108]. Moreover, ligand modified nanoparticles further enhance cellular uptake by receptor-mediated endocytosis [109, 110]. Therefore, nanoparticles have attracted attention as therapeutic drug vehicles for improved disease diagnosis and treatment [105, 111, 112].

SELP nanoparticles, which have potential to serve as stimuli responsive drug delivery vehicles, can be prepared using a variety of methods, including direct self-assembly [25], and gold nanoparticle triggered [69] or hydrophobic drug triggered self-assembly [23] in aqueous solution. The self-assembly of SELPs in aqueous solution is based on hydrogen bonding between the silk blocks, and the hydrophobicity differences between silk and elastin blocks. Xia *et al.* demonstrated that silk blocks tend to self-assemble into the core of micellar-like SELP nanoparticles, and the average radius of the nanoparticles can be controlled by the silk-elastin ratio [25]. Lin *et al.* utilized nickel-chelate-nitrilotriacetate (NTA-Ni²⁺) functionalized gold nanoparticles to trigger the formation of core/shell gold/SELP nanoparticles based on the nickel affinity of the polyhistidine tag fused at the N-terminus of SELP [69]. Xia *et al.* also demonstrated that the self-assembly of SELPs can be triggered by adding hydrophobic molecules to form the core of micellar-like nanoparticles [23]. Biodistribution of drug loaded nanoparticles depends on the physicochemical properties, especially size. The diameter of nanoparticle therapeutics for cancer should be in the range of 10–150 nm to cross the endothelial barrier [16, 105, 113]. The nanoparticles formed by SELPs using the above methods are in the range from 20 nm to 150 nm (Table 2). Therefore, the SELP nanoparticles have potential to be used as drug delivery vehicles.

Because the stimuli responsive elastin-like blocks in SELPs, the nanoparticles tend to respond to a variety of environmental stimuli to achieve targeted delivery of SELP nanoparticle therapeutics. Figure 2 demonstrates the thermal response of gold/SELP core/shell nanoparticles formed by SELPs named S4E8G with various molecular weights (18 – 90 kDa). At 25°C, below the inverse transition temperature, Au-S4E8G nanoparticles are dispersed in aqueous solution (Figure 2a). The size of the gold/SELP nanoparticles was about 30 nm with an Au core diameter of 12 nm and a uniform SELP coating of 13 nm at 25°C [69]. When heated to 60°C, above the inverse transition temperature, Au-S4E8G nanoparticles tend to aggregate into larger particles (Figure 2b) due to the hydrophobic effect. The apparent size changes of the gold/SELP nanoparticles were confirmed by dynamic light scattering (Figure 2c). Local hyperthermia increased accumulation and prolonged retention of thermal responsive nanotherapeutics in tumors. Taking advantage of the thermal response of SELPs, in the form of aggregates, mild local hyperthermia can be applied to achieve tumor targeting in addition to passive targeting through the EPR effect [21, 87].

Due to the attractive stimuli responsive features and self-assembly properties of SELP nanoparticles, the applicability of genetically engineered SELP nanoparticles as drug carriers was evaluated. Figure 3 demonstrates the delivery of doxorubicin into HeLa cells via SE8Y nanoparticles at different time points: (a) 40 min and (b) 4 h by confocal laser scanning microscopy using free doxorubicin as the control. At 40 min of incubation, free

doxorubicin accumulated in cell nuclei, while doxorubicin loaded SELP nanoparticles (S1/Dox) mostly accumulated in the cytoplasm. Longer incubation of S1/Dox with HeLa cells facilitated the diffusion of doxorubicin from the SELP nanoparticles into the nuclei. Laser scanning microscopy images of HeLa cells treated with S1/Dox and free doxorubicin indicated the different uptake pathways of nanoparticles and free doxorubicin [23]. Notably, S1/Dox and S2/Dox showed higher cytotoxicity than the free drug, which might be explained by the controlled release of drugs leading to a higher concentration of doxorubicin entering the nuclei.

5. Silk-elastin-like protein hydrogels for gene and virus delivery

Gene therapy is a promising approach for the treatment of various diseases such as cancers. The principle is based on the delivery of therapeutic genes into target cells in the patient by either using naked DNA or a viral vector delivery. Current attempts to improve safety and efficacy of gene delivery focus on polymeric matrix-controlled gene delivery systems bearing the advantages of controlled DNA release profiles, localized delivery to particular tissue and the protection of DNA from degradation [114].

As genetically engineered polymers, SELPs can be good candidates as polymeric matrices for gene delivery. Temperature sensitive SELPs, which are liquid at room temperature and form hydrogels once injected in the body, were studied and evaluated in detail to serve as gene delivery matrices. Figure 4 depicts the scheme of injectable SELP hydrogel formation for controlled release of DNA. Early in the 2000s, Gandheri *et al.* demonstrated the potential of recombinant SELP-hydrogels for controlled gene delivery in an elaborate study on DNA and adenoviral vector delivery using SELP-47K hydrogels [115]. Comparing plasmids of different molecular weights from 2.6 kbp (pUC18) to 11kbp (pFB-ERV), it was found that the average release and effective diffusivity was inversely related to the molecular weight of the plasmids. The influence of plasmid conformation on the rate of release showed the trend in descending order of linear > supercoiled > open circular. Embedded DNA and adenoviral particles were bioactive after 28 and 22 days of incubation, respectively, and *in vivo* experiments using an athymic nude mouse/MDA-MB-435 breast cancer model showed greater gene expression compared to naked DNA [115].

The binding/releasing mechanism of DNA to SELPs, based on ion-exchange, was discovered when studying the influence of ionic strength of the media [114]. At pH 7.4, primary amines of lysine and arginine residues are protonated and show interactions with the negatively charged phosphates of DNA. With increasing ionic strength of buffer, the concentration of counter-ions increases, weakening the interaction between the DNA-phosphates and the amino-groups, leading to the release of the previously ionically bound DNA [114]. Since these studies, different parameters have been examined, such as SELP and DNA concentration, SELP silk-elastin ratio and molecular weight, SELP hydrogel cure time, which influence the release of DNA and adenovirus *in vitro* and *in vivo*. For DNA as well as for virus delivery, the release was inversely correlated to concentration of SELPs and SELP hydrogel cure time [114, 116–118]. Increased concentration of the polymer led to an increase of the density prevented loss of embedded DNA or virus. Studies in the presence of elastase showed increased swelling ratio of the polymer with regard to enhanced release of

DNA [118]. Comparing different ratios of silk to elastin and different lengths of the SELPs showed that higher elastin content led to faster degradation [73, 117–120]. For SELP-415K, SELP-47K, SELP-815K (the 1st number stands for silk-monomer, 2nd number for elastin monomers, K for lysine residue in variable position), the SELP-815K at 4 wt% polymer concentration showed the highest gene expression and anticancer response when tested with adenovirus in tumor bearing mouse models [73, 119]. An assessment of the toxicity of SELP-815K-mediated viral delivery in healthy non-tumor-bearing mice showed reduced systemic toxicity compared to free viral injection [121]. Additionally, when comparing SELP-815K with polaxamer 407, a FDA approved commercially available synthetic tri-block co-polymer of poly(oxyethylene) and poly(oxypropylene), SELP-815K supported the greater average tumor size reduction, longer time to tumor rebound and greater survivability [120].

These studies of gene/virus delivery using SELPs as the polymer matrix showed promising results. Interestingly, to date, no research studies were performed to evaluate the application of SELPs for targeted drug delivery by combining them with tumor homing peptides (THPs). Although genetic engineering would give the opportunity to enable functionalization by incorporating a specific peptide sequence. Bioengineered silk was a good candidate for the modification with tumor homing peptides. THPs integrated into recombinant silk protein led to a significant enhancement of target specificity of nanoscale silk-pDNA complexes to tumor cells combined with low toxicity [122–124]. Recently drug loaded spheres from hybrids composed of THPs and bioengineered silk worked well as targeted drug delivery devices [19]. Applying these findings to SELPs should offer additional options for targeted drug/gene/virus delivery.

6. Biocompatibility of silk-elastin-like proteins

Biocompatibility is a critical factor governing the utility of biomaterials in drug delivery. Low cytotoxicity drug delivery vehicles are desired for controlled delivery of therapeutics [23, 108]. *Bombyx mori* silk, a FDA approved biocompatible material, and elastin, a major component of the extracellular matrix, cause little immunogenicity, cytotoxicity or inflammation [55, 125–127]. A number of studies demonstrated that SELPs, consisting of the representative sequences of silk and elastin, are biocompatible [23, 39, 40, 65, 128, 129]. The *in vitro* cytotoxicity of SELP nanoparticles against Hela cells was investigated by Xia *et al.* [23]. Cell viability was above 90% when the concentration of SELPs (SE8Y, S2E8Y and S4E8Y) was increased to 200 µg/mL (Figure 5a). Therefore, SELP nanoparticles exhibit low cytotoxicity to go with their potential as chemotherapy drug carriers. Ozaki *et al.* studied the effect of SELPs on the migration, proliferation, and protein production from L929 mouse fibroblasts [40]. Upon culturing with different concentrations of SELPs, cell migration and collagen production were significantly enhanced with SELP concentrations from 10⁻³ to 10 µg/ml. These studies suggested that the SELPs exhibit low cytotoxicity, and they have potential to promote the migration of fibroblasts and macrophages, and fibroblast collagen production. *In vitro* and *in vivo* biocompatibility of SELP electrospun fiber scaffolds [65] and injectable hydrogels were also confirmed [128]. NIH/3T3 fibroblast cell viability and proliferation were observed on SELP-47K scaffolds [31] and no signs of immunological

reactivity or chronic inflammation were observed over 28 days for injectable SELP-47K hydrogels [97].

7. Conclusions

The genetically engineered SELPs provide a new class of biopolymers with potential utility in controlled delivery of small molecules, DNAs and genes. The bioengineering approach utilized for their synthesis provides ease of rational design and control over structure at the molecular level to expand the versatility of SELPs for targeted delivery. Modulation of SELP properties can be achieved through precise changes in sequence, silk-elastin ratios and molecular weight. SELPs can be aqueously processed into a diverse range of biomedical material formats, including nanoparticles, films, thin coatings, hydrogels, and scaffolds. The excellent material properties, unique and versatile processing options, biocompatibility, biodegradability, and stimuli responsive and highly tunable material properties, suggest that SELPs are poised to significantly impact the field of drug delivery and injectable controlled release, but also many other areas of biomedical applications, including tissue engineering, stimuli responsive thin coating, biosensors and bio-optics.

8. Expert opinion

Genetically engineered proteins provide a high degree of molecular definition for correlating chemistry and structure with function in controlled release. Through appropriate changes (e.g., mutations) in the genetic template based on sequence-structure-function relationships, the properties and functionalities of genetically engineered proteins can be modified and optimized for the need of specific biomedical applications. In contrast to conventional synthetic polymers, the sequence and molecular weight of genetically engineered proteins can be strictly controlled by genetic templates, leading to monodisperse, precisely defined biopolymers. This level of control provides unprecedented insight into the role of such features on delivery and targeting, avoiding the otherwise less defined results with polydisperse polymers. Moreover, genetically engineered proteins degrade *in vivo* into non-toxic small peptides, while synthetic polymers may not degrade or can sometimes degrade into toxic by-products, such as N-isopropylacrylamide (NIPAAm) monomers. Therefore, genetically engineered proteins have attracted great attention for controlled drug delivery.

Prior to the studies of SELPs, several other classes of recombinant proteins, such as ELPs, SLPs and CLPs, were synthesized and found to have interesting self-assembling or stimuli responsive properties. Studies have demonstrated that ELPs undergo a sharp disorder to order structural transition upon exposure to environmental triggers. These proteins are soluble in aqueous solution below their inverse transition temperature, T_t , while they aggregate and become insoluble when the temperature is raised above the T_t . In addition to the change in temperature, inverse temperature transitions can be induced by changes in pressure, ionic strength, pH, electric field and redox conditions. SLPs are designed to form beta sheet crystalline structures in their alanine rich regions and these proteins self-assemble into nanoparticles for drug delivery. CLPs form triple helices and self-assemble into fibers. Among the different types of recombinant proteins studied, SELPs combine the outstanding physical and biological properties of silk and elastin, offering utility in comparison to

alternatives due to the versatility in design, tunability of physical properties and biocompatibility.

SELPs are emerging biomaterials for targeted drug delivery because of their self-assembling and stimuli responsive properties. SELPs self-assemble into nanoparticles in the size range of 20 – 150 nm in aqueous solution, with the specific size dictated by the molecular weight and sequence chemistry. These nanoparticles can passively accumulate in tumor tissues due to the high vascular permeability and poor lymphatic drainage of tumors, a phenomenon known as the EPR effect. SELPs can also actively target tumor sites due to their environmental sensitivity. The stimuli responsive properties of SELPs are governed by both the silk and elastin sequences; in particular by the silk-elastin ratio, the X residue in the elastin-like blocks, and the overall protein chain length. Taking advantage of the temperature responsive properties, SELPs can accumulate in tumor tissues through the application of local hyperthermia. Mild local hyperthermia can induce the aggregation of SELPs to form insoluble proteins in the heated areas, therefore located at the tumor site. The current applications of SELPs have focused mostly on thermally targeted delivery of therapeutics. Further biomedical applications that utilize other stimuli triggers of SELPs for targeted delivery, including pH, ionic strength, redox, enzymatic stimuli and electric field, are in progress. Upon fully exploiting this range of stimuli responsive features of SELPs, a broader range of SELP functional biomaterials can be generated and utilized towards controlled therapeutics release or for tissue regeneration.

SELPs are good candidates as polymeric matrices for controlled gene release. Temperature sensitive SELPs are liquid at room temperature and form hydrogels once injected in the body. SELP hydrogels have been studied as gene delivery systems, and showed promising results. However, the hydrogels formed from SELP to date are irreversible. It would be interesting to incorporate stimuli sensitive motifs or biorecognition motifs into these hydrogels for reversibility.

Earlier studies have shown biocompatibility of SELPs both *in vitro* and *in vivo*. No signs of immunological reactivity or chronic inflammation were observed over 28 days for an injectable SELP hydrogel, named SELP-47K. The biocompatibility of newer SELPs remains to be evaluated. Further, longer term outcomes in terms of degradation and metabolism/distribution *in vivo* would help to further refine the utility of these protein-based materials.

In summary, the specific and useful properties of SELPs make them superior candidates for biomedical applications, such as nano-devices, biosensors, bioseparations, tissue engineering, and most prominently, targeted drug delivery for small molecule drugs, proteins and genes. The work highlighted in this review provides an important path forward in the further elucidation of the influence of protein design on physicochemical properties of materials generated from these proteins, leading to the development of future generations of smart drug delivery systems for controlled release in a complex physiological environment. The precise tailorability (size, chemistry, sequence), the evolving understanding of sequence-assembly properties, and the modulation of structure and thus function for these types of proteins, combined with already-demonstrated tumor targeting and the vast options for further chemical modifications, point to these protein systems as key polymers in

elucidating specific design roles related to optimizing tumor targeting, as well as for viable delivery systems for future clinical needs.

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Article highlights

- SELPs, consisting of semicrystalline silk-blocks and elastomeric elastin-blocks, are useful for controlled delivery due to their rational design, tunable structure-function, biocompatibility, degradability and target specificity.
- SELPs respond to various environmental triggers including temperature, pH, ionic strength, redox, enzymatic stimuli and electric field.
- Modulation of stimuli responsive properties of SELPs can be achieved through changes in the X residue in the elastin-like blocks, silk-elastin ratios and molecular weight.
- SELPs can be aqueously processed into a diverse range of biomedical material formats, including nanoparticles, films, thin coatings, hydrogels, and scaffolds.
- –Temperature responsive drug loaded SELP nanoparticles used as delivery vehicles for chemotherapeutics suggested different drug uptake pathways than free drug.
- Injectable SELP hydrogels can be used for gene and virus delivery with improved safety and efficacy.
- SELPs are biocompatible materials with low cytotoxicity *in vitro* and *in vivo*.

Figure 1.
General bio-synthesis scheme of genetically engineered proteins using recombinant DNA technology.

Figure 2.

Thermal responsive gold/SELP core/shell nanoparticle assembly. (a) TEM image of dispersed gold/SELP nanoparticles at 25°C, (b) TEM image of gold/SELP nanoparticle aggregation formed at 60°C, and (c) the reversible, temperature dependent apparent size changes of gold/SELP nanoparticles in PBS buffer measured by dynamic light scattering. Reproduced with permission from [69]. Copyright 2014 American Chemical Society.

Figure 3.

Anticancer chemotherapeutics delivery by SELPs. Doxorubicin delivery into HeLa cells using SELP nanoparticles at (a) 40 min and (b) 4 h incubation time. Key: blue signal - nucleus stained with DAPI; red signal - doxorubicin; scale bar - 10 μm. (c) In vitro cytotoxicity and (d) IC₅₀ values of doxorubicin loaded SELP nanoparticles, S1/Dox, S2/Dox, and S4/Dox, and free doxorubicin against HeLa cells.

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Figure 4.

Scheme of injectable SELP hydrogel formation for controlled DNA release: (1) mixing plasmid DNA and SELP solution at room temperature, (2) temperature responsive SELP forming a hydrogel at body temperature.

Figure 5. Biocompatibility of SELPs: cytotoxicity of three different SELPs with various silk-elastin ratios, termed S1, S2 and S4, against Hela cells. Reproduced with permission from [23]. Copyright 2014 American Chemical Society.

Table 1

Stimuli responsive features of SELP-based dynamic protein polymers.

X residue in elastin block	Stimuli	Ref.
<i>Phe, Ile, Tyr, Gly</i>	Temperature Ionic Strength	[25, 32, 35, 103]
<i>Glu, Lys</i>	pH Electrical Field	[35, 104]
<i>Cys</i>	Redox	[35]
<i>RGYSLG</i>	Phospho/dephosphorylation	[35]

Data taken from [35]

Table 2

Sequence and size of SLP nanoparticles.

Name	Mw (kDa)	Silk block	Elastin block	Size ^d (nm)	Ref.
SE8Y	56	GAGAGS	GVGVP or GYGVP	40 ± 13 (20°C) ^b 220 ± 37 (60°C) ^b	[25]
S2E8Y	53	GAGAGS	GVGVP or GYGVP	38 ± 14 (20°C) ^b 174 ± 28 (60°C) ^b	[25]
S4E8Y	48	GAGAGS	GVGVP or GYGVP	68 ± 12 (20°C) ^b	[25]
S4E8G 3mer	18	GAGAGS	GVGVP or GGGVP	~25 (25°C) ^c ~60 (60°C) ^c	[69]
S4E8G 8mer	42	GAGAGS	GVGVP or GGGVP	~28 (25°C) ^c ~80 (60°C) ^c	[69]
S4E8G 13mer	66	GAGAGS	GVGVP or GGGVP	~32 (25°C) ^c ~100 (60°C) ^c	[69]
S4E8G 18mer	91	GAGAGS	GVGVP or GGGVP	~41 (25°C) ^c ~160 (60°C) ^c	[69]
SE8Y/DOX	56	GAGAGS	GVGVP or GYGVP	50 ± 10 (25°C) ^b	[23]
S2E8Y/DOX	53	GAGAGS	GVGVP or GYGVP	72 ± 141 (25°C) ^b	[23]
S4E8Y/DOX	48	GAGAGS	GVGVP or GYGVP	142 ± 10 (25°C) ^b	[23]

^a Size measurement temperatures are specified in the parentheses

^b R_h hydrodynamic radius

^c Diameter