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Elastin-Like Polypeptides for Biomedical Applications

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Abstract

Elastin-like polypeptides (ELPs) are stimulus-responsive biopolymers derived from human elastin. Their unique properties—including lower critical solution temperature phase behavior and minimal immunogenicity—make them attractive materials for a variety of biomedical applications. ELPs also benefit from recombinant synthesis and genetically encoded design; these enable control over the molecular weight and precise incorporation of peptides and pharmacological agents into the sequence. Because their size and sequence are defined, ELPs benefit from exquisite control over their structure and function, qualities that cannot be matched by synthetic polymers. As such, ELPs have been engineered to assemble into unique architectures and display bioactive agents for a variety of applications. This review discusses the design and representative biomedical applications of ELPs, focusing primarily on their use in tissue engineering and drug delivery.

Contents

INTRODUCTION

Beyond their role as the nanomachines of living things, proteins are increasingly being used as materials in medical applications. Advances in genetic engineering have enabled exploration of protein structure and function, leading to the creation of biologically inspired materials for diverse biomedical applications that span tissue engineering, drug delivery, biosensors, and theranostics (1). Protein-based materials can now be designed to mimic biological structures such as collagen and exosomes (2, 3), display bioactive molecules, or respond to medically relevant stimuli.

One class of biologically inspired, protein-based materials extensively explored for such applications is elastin-like polypeptides (ELPs). These biopolymers are based on the sequence of elastin, an extracellular matrix protein that provides elasticity to tissues such as arteries, lungs, and skin (4). Biomaterials that mimic elastin have been studied extensively owing to elastin's unique properties (5). Elastin's soluble precursor, tropoelastin, consists of alternating hydrophobic and hydrophilic cross-linking domains (6). ELPs are composed of repeat units of a Val-Pro-Gly-X-Gly motif derived from the hydrophobic domain of tropoelastin (7), where X represents a guest residue that can be any amino acid except proline. ELPs are thermally responsive and demonstrate lower critical solution temperature (LCST) phase behavior. Below a characteristic transition temperature (T_t), an ELP is soluble; above the T_t, an ELP reversibly phase separates into an insoluble, polymer-rich, coacervate phase (8). This phase behavior can be controlled through selection of guest residue and chain length (9), and the behavior is retained when the ELP is fused to a peptide or small molecule.

In addition to their phase behavior, ELPs have several advantages over synthetic polymers. As ELPs are genetically encoded, they can be recombinantly synthesized with complete control of polymer length and sequence to produce a monodisperse population, an impossible outcome for synthetic polymers (10). Monodispersity is desirable for many biomedical applications. For example, in drug delivery, polymer molecular weight impacts plasma half-life, biodistribution, and

clearance rate and must hence be defined with the greatest possible precision (11, 12). Second, ELPs can be customized with biofunctional groups at precise locations within the sequence. Peptides with reactive side chains are easily incorporated into the ELP sequence, either at the polypeptide termini or between pentapeptide repeats. Drugs, ligands, imaging agents, and cross-linking sites can be added to ELPs without compromising their thermally responsive behavior (13, 14). Lastly, as ELPs are composed of amino acids, they are biodegradable and have nontoxic byproducts (15).

The fine control over ELP sequence and length allows precise manipulation of ELPs' biological activity, chemical reactivity, and physical properties, including their stimulus-responsive and self-assembly behavior, making them attractive for biomedical applications. In this review, we focus on the design and synthesis of ELPs, ELP fusions, and ELP-based materials in which the mechanical and biochemical properties of ELPs are enhanced for tissue engineering and drug delivery applications (see **Figure 1**).

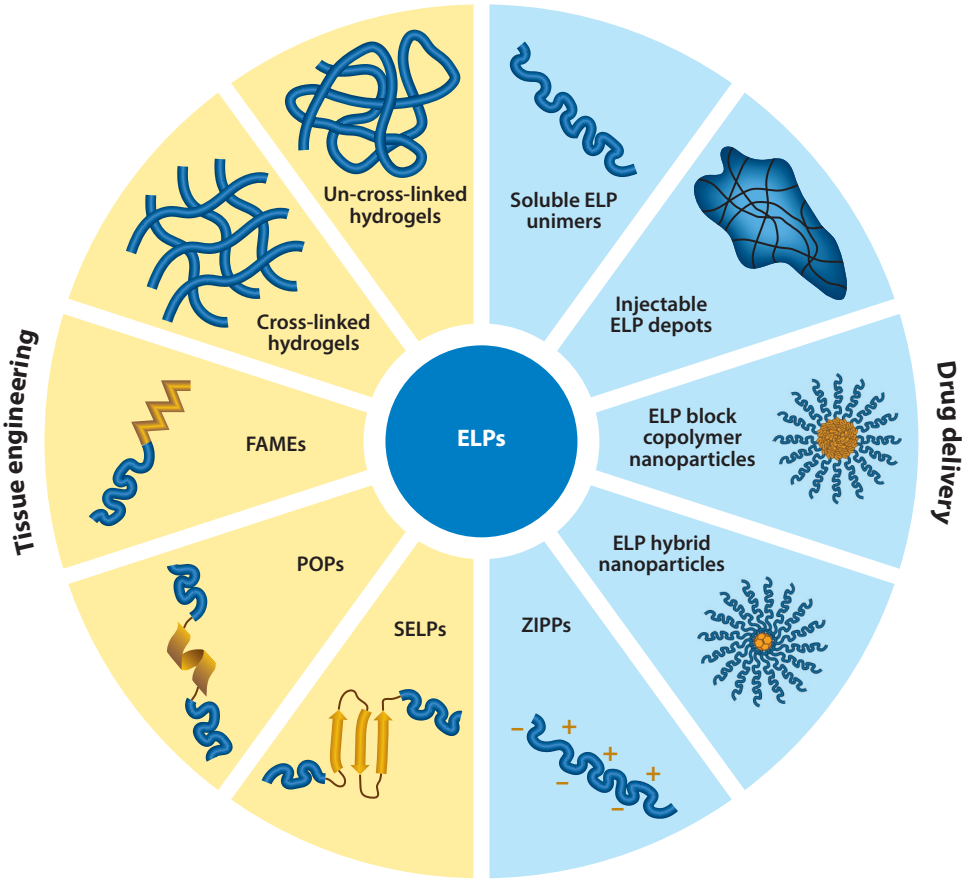

Figure 1

ELP-based materials that have been engineered for tissue engineering (*yellow background*) and drug delivery (*blue background*) applications. Abbreviations: ELP, elastin-like polypeptide; FAME, fatty acid–modified ELP; POP, partially ordered polymer; SELP, silk ELP; ZIPP, zwitterionic polypeptide.

ENGINEERING ELPs

ELP Synthesis

ELPs are recombinant, unstructured proteins that are typically expressed in *Escherichia coli* (10, 16, 17). Owing to their highly repetitive sequence, the genes for ELPs cannot be assembled using standard methods for gene assembly that rely on overlapping sequence homology or hybridization (18). Polymerase chain reaction has historically resulted in poor priming due to the high guanine-cytosine content of ELP genes, leading to polydisperse gene products. Thus, alternative gene assembly methods have been developed to address this bottleneck.

An early technique used to synthesize ELPs was concatemerization (19), in which ELP monomer genes are ligated at overlapping sticky ends to create an oligomer within a vector. This method is simple and fast, but ligation with the parental plasmid can result in uncontrolled oligomerization (20, 21). Recursive directional ligation was developed by Meyer & Chilkoti (22) to address this issue by introducing an ELP gene into a linearized vector; each step adds only one oligomer, producing a gene with uniform length. However, linearization of the parent vector with a single restriction enzyme can result in self-ligation, reducing oligomer insertion and gene synthesis efficiency (22, 23). Recursive directional ligation by plasmid reconstruction, developed by McDaniel et al. (24), prevents self-ligation by introducing a second cut site into two ELP-encoding parental plasmids (**Figure 2**). Using this technique, a functional plasmid is produced only by proper ligation of the ELP-encoding halves.

ELP Purification

The temperature responsiveness of ELPs can be exploited to allow inexpensive purification of ELPs without chromatography using a technique called inverse transition cycling (ITC),

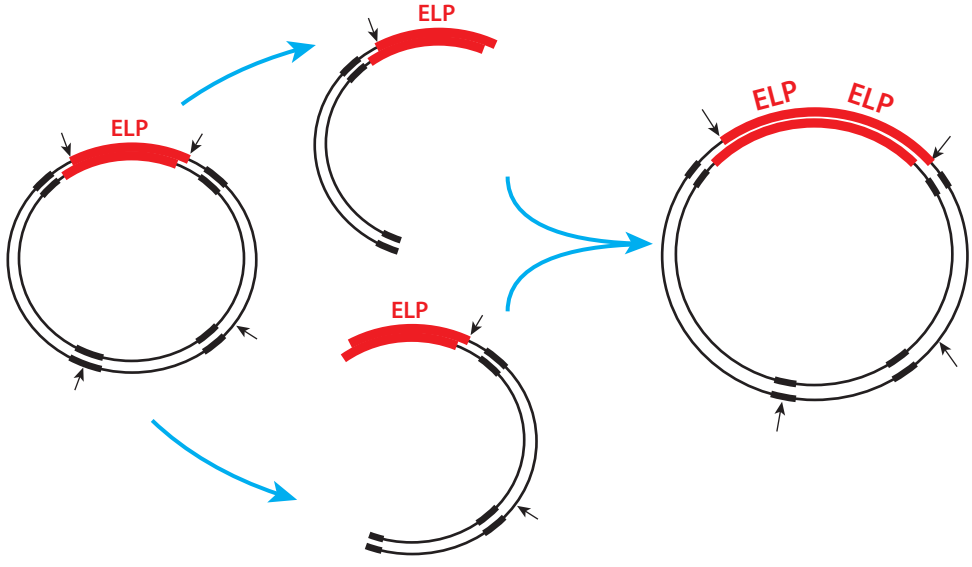

Figure 2

Recursive directional ligation by plasmid reconstruction. The elastin-like polypeptide (ELP)-containing plasmid is digested with AcuI and BglI or BseRI and BglI. The compatible plasmid pieces are ligated together to double the length of the ELP. Figure adapted with permission from Reference 24; copyright 2010 American Chemical Society.

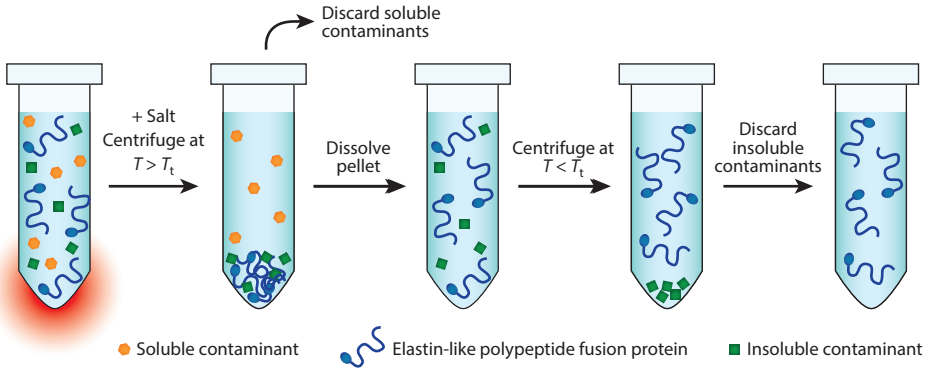

Figure 3

Purification of elastin-like polypeptides (ELPs) and ELP fusions by inverse transition cycling. After cell lysis, ELP coacervation is triggered with the addition of heat and salt. Soluble contaminants are separated from the ELPs by a centrifugation step at a temperature above the transition temperature ($T > T_t$). After the ELP is redissolved in cold buffer, a centrifugation step at $T < T_t$ is performed to remove insoluble contaminants from the ELPs, which are now in solution.

developed by Meyer & Chilkoti (25) in 1999 (**Figure 3**). ITC involves three main steps. First, cells expressing ELP are lysed, and the lysate is centrifuged below the ELP T_t—a temperature at which the ELP is soluble—to pellet and remove insoluble cellular debris. Second, the supernatant—containing soluble ELP—is collected and heated above the ELP T_t, and the suspension is centrifuged to pellet the aggregated ELP. Third, the ELP pellet is dissolved by adding cold buffer followed by centrifugation at a temperature below the T_t to remove any insoluble contaminant proteins, leaving the purified ELP in solution (25, 26).

ELPs can also be used as a tag to purify other proteins, as ELP fusions retain the inverse transition behavior of the ELP. ELP fusions can be purified using ITC, and the ELP can then be removed using a proteolytic cleavage site or a self-cleaving intein—a self-splicing protein domain—that has been inserted between the ELP and target protein (27–29). Chilkoti and others have developed several approaches to cleave the ELP from its fusion partner. The first, most straightforward approach involves the incorporation of a peptide sequence between the ELP and protein (30–32), which can be cleaved by protease that recognizes and cleaves this sequence to liberate the ELP from the protein. The second approach involves the introduction of an intein between the protein and ELP that cleaves itself out upon the addition of a reducing agent such as dithiothreitol (33, 34). The third, somewhat counterintuitive method involves the introduction of a sortase A recognition site—typically a Leu-Pro-Glu-Thr-Gly motif—that is recognized by the enzyme in the presence of triglycine and cleaves between the threonine and glycine residues and ligates the triglycine to the threonine cleavage (35, 36). The sortase A cleavage method is useful not just for cleavage of the ELP from its fusion partner but also for concurrent conjugation of an extrinsic moiety of interest, such as a fluorophore, imaging agent, or polymerization initiator (36, 37). Protein purification using ELP tags and genetically encoded cleavage sites can be scaled and multiplexed to allow high-yield protein production using commonly available labware and instrumentation (32).

ELP Characterization

After expression and purification, ELPs must be characterized to ensure their biophysical characteristics and phase behavior are suitable for each application. Matrix-assisted laser desorption/

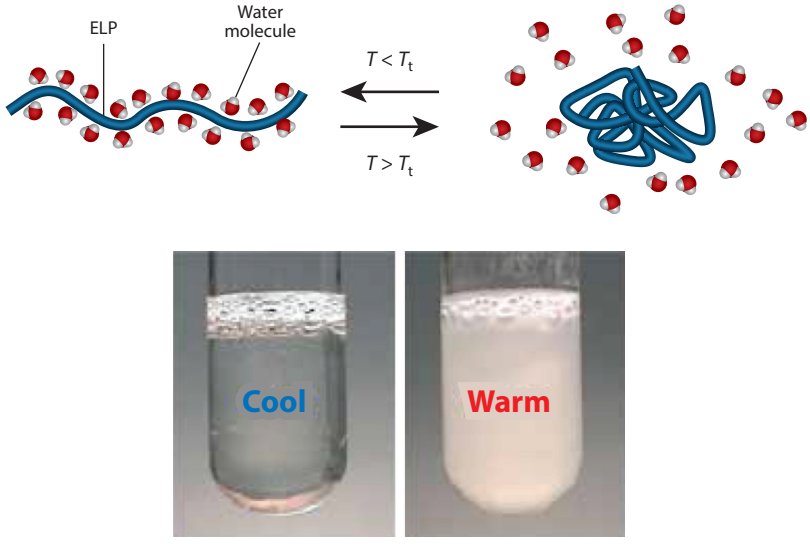

Figure 4

Lower critical solution temperature (LCST) behavior of elastin-like polypeptides (ELPs). At temperatures below its transition temperature (T_t), the ELP is hydrated and fully soluble, appearing as an optically transparent solution. As the temperature is raised above the T_t of the ELP, the ELP coacervates and phase separates, appearing as a cloudy solution. As the temperature is lowered below the T_t of the ELP, the ELP re-solubilizes.

ionization mass spectrometry can be used to determine their molecular weight and ensure the construct was expressed without truncation. High-performance liquid chromatography and gel electrophoresis should be used to evaluate their purity (38). Bioactive peptides and protein domains fused to an ELP must be characterized using in vitro assays to verify that they retain their activity (17). Tissue engineering applications may require characterization of ELP material stiffness and elasticity by mechanical testing (39–41). Finally, the endotoxin level of all ELPs and their fusions must be measured to ensure that they are below the limit specified by the US Food and Drug Administration. In the event that the residual endotoxin level is not below this limit, endotoxin can be removed by polymyxin chromatography or ion exchange chromatography (42). Furthermore, the biocompatibility of ELPs and ELP fusions should be evaluated when appropriate to determine whether cells adhere to and proliferate on the material.

The most unique characteristic of ELPs is their thermal responsiveness. ELPs display LCST phase behavior, an entropically driven phenomenon that causes the polypeptide solution to become insoluble above its T_t (9, 43) (**Figure 4**). This behavior is due to an unfavorable entropy of mixing, as water molecules along the polypeptide chain are highly ordered (44). Consequently, the entropy term for this interaction is negative. At low temperatures, the Gibbs free energy change is negative, allowing spontaneous mixing of ELP and solvent. As temperature increases, mixing becomes more energetically unfavorable, causing the ELP to phase separate from water (45, 46). The temperature at which this phase separation occurs depends on several factors. First, the T_t of an ELP decreases as the molecular weight of its concentration in solution increases (47). The T_t is also impacted by the hydrophobicity of the guest residue in its pentapeptide motif. More hydrophobic guest residues result in a lower T_t, while more hydrophilic residues increase the T_t (47). Furthermore, the fusion of an ELP with a peptide or protein can impact its T_t. The degree

to which the T_t is affected is largely attributed to the surface hydrophobicity of its fusion partner, with more hydrophobic proteins depressing the T_t (13).

The ELP T_t can be quantified by measuring the optical turbidity of an ELP solution at 350 nm (OD350) as a function of temperature. Within a narrow temperature window (1–2°C), the ELP solution undergoes a transition from an optically transparent solution to an opaque mixture due to ELP aggregation (14). A plot of OD350 versus temperature displays a sigmoidal curve, where T_t is indicated by the inflection point of the curve. The T_t is concentration dependent and must be determined over a range of ELP concentrations relevant to the application.

TISSUE ENGINEERING

ELPs are attractive for tissue engineering owing to their customizable physical properties and biocompatibility. Unlike nonbiological synthetic polymers, the ELP molecular weight and sequence can be precisely controlled (48, 49). Cell recognition and cross-linking sites can be introduced into the sequence to tune cell adhesivity and mechanical properties to mimic native biological substrates. Swelling, degradation, and viscoelastic properties can be modified by synthesizing ELP block copolymers of alternating hydrophobic and hydrophilic blocks and by incorporating cross-linking domains (48). Additionally, ELPs can be expressed at high levels—up to several hundred milligrams per liter in shaker flask culture and up to a few grams per liter in high-density fermenters—a useful attribute for tissue engineering that often requires gram-scale quantities of material (32, 48, 50). Despite their more complex architecture, ELP block copolymers exhibit LCST phase behavior and can hence be purified without chromatography by exploiting their phase behavior (25). Their LCST phase behavior can be further exploited to create injectable formulations for applications where a void or defect must be filled. Upon injection in vivo, an ELP solution with a T_t below 37°C forms an insoluble coacervate and fills the defect (48) (**Figure 5**). Another useful attribute of ELPs for tissue engineering and regenerative medicine applications is that ELPs degrade in vivo into nontoxic amino acids and are typically nonimmunogenic (7, 10).

ELPs have been used for cartilage regeneration and vascular graft, liver, and ocular tissue engineering (48, 51). Different ELP architectures and recombinant or chemical modifications have been explored to create ELPs with mechanical and biochemical properties that promote tissue

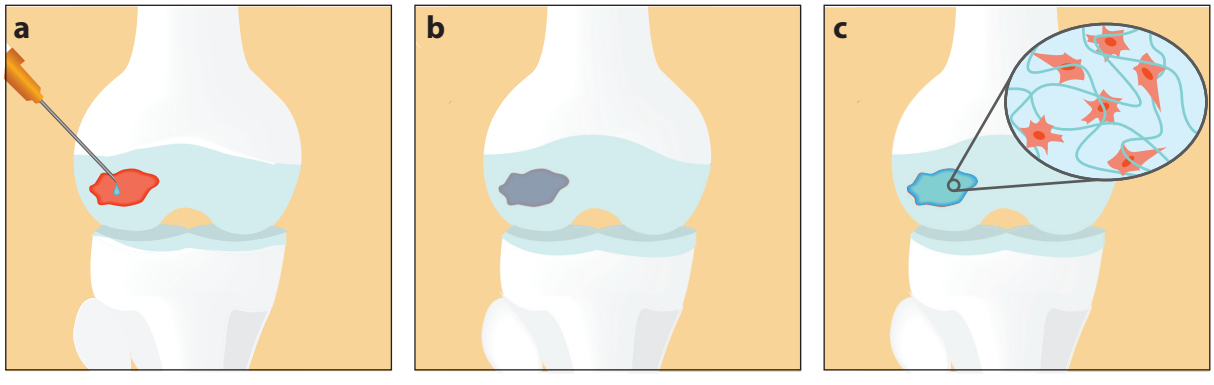

Figure 5

Application of elastin-like polypeptides (ELPs) in tissue engineering to fill voids to repair cartilage defects. (*a*) The ELP is injected as a solution and takes on the void shape before undergoing its phase transition (*b*) to form a viscous gel. External stimuli such as irradiation with ultraviolet light can be used to cross-link the ELP. (*c*) Upon gel formation, cells infiltrate the material.

repair. In this section, we discuss several types of ELP-based materials developed for tissue engineering and regenerative medicine applications.

ELP Hydrogels

When a material is designed for tissue engineering, the mechanical properties of the material must match those of the native tissue. This is particularly important in cartilage repair, for which restoring the mechanical properties and function of the cartilaginous material is crucial to maintain tissue and joint function. Early studies by Chilkoti and coworkers (e.g., 52) demonstrated that an un-cross-linked ELP that coacervates at 35°C had rheological properties similar to those of collagen and hyaluronan. In cell culture, chondrocytes were entrapped in the ELP solution and were cultured as a monolayer. These cells and the matrix they produced were collected by reversing the ELP phase behavior and gently agitating the culture plates. After 4 weeks, type II collagen and sulfated glycosaminoglycan had accumulated in the wells, indicating that the ELP supported the chondrocyte phenotype (52). This work was expanded by demonstrating that ELPs support articular cartilage extracellular matrix formation by other medically relevant cell types and in other cell culture conditions (53, 54). For example, human adipose-derived adult stem cells were successfully cultured on an ELP coacervate, and chondrogenesis occurred in the absence of chondrogenic supplements and in low-oxygen conditions (53).

Cross-Linked ELP Hydrogels

The studies summarized above demonstrated the potential of using ELPs as in situ scaffolds for cartilage repair. However, un-cross-linked, coacervated ELPs have limited mechanical strength and cannot support the loads experienced by cartilaginous tissue in vivo (52). The dynamic shear stiffness of cartilage ranges from 100 to 500 kPa, nearly four orders of magnitude greater than that of an un-cross-linked ELP coacervate. Thus, cross-linked ELPs have been developed to increase their dynamic stiffness.

Urry et al. (55) created cross-linked ELP depots using γ-irradiation, resulting in nonspecific cross-linking through free radical formation. Although there was no control over the degree or location of cross-linking, the ELP maintained its temperature-dependent phase transition behavior. An Arg-Gly-Asp-Ser cell adhesion sequence was subsequently introduced into the ELP backbone to produce a gel-like cell adhesive matrix. At an Arg-Gly-Asp-Ser:ELP ratio $\geq$60, cell adhesion was similar to that of cells on adsorbed fibronectin. In contrast, at an Arg-Gly-Asp-Ser:ELP ratio of $<$60, cell adhesion and spreading were reduced, indicating that the native ELP sequence did not support cell adhesion. This study established the potential for ELPs in tissue engineering by demonstrating that they can incorporate cell recognition sequences and promote cell adhesion (56).

McMillan & Conticello (20) added controlled, chemoselective cross-linking into ELP coacervates by introducing a lysine residue every six repeats and using a homobifunctional amine cross-linker to cross-link the lysines. Organic solvent was used for the cross-linking reaction to prevent ELP phase transition and promote more uniform hydrogel formation, and the resulting cross-linked ELP also showed inverse transition behavior in aqueous solvent. Chilkoti and coworkers (57) expanded on this work. In an effort to avoid the use of organic solvents and cytotoxic cross-linkers, Chilkoti and coworkers (58) used a nontoxic, trifunctional cross-linker, β-[tris(hydroxymethyl)phosphino] propionic acid (THPP) (betaine) that allows cross-linking in aqueous solvents. They applied this strategy to treating osteochondral defects in goats: ELP and THPP solution were injected into a joint defect, and the ELP was allowed to cross-link in situ.

After 3 months, the ELP matrix showed cell infiltration and matrix synthesis, although some scaffold degradation occurred (59).

Enzymatic cross-linking is an alternative approach that can be carried out in water and is cytocompatible. McHale et al. (50) produced hydrogels using enzymatic cross-linking of ELPs with periodic glutamine and lysine residues that were spaced every seven Val-Pro-Gly-X-Gly repeats, using the tissue transglutaminase. The tissue transglutaminase–cross-linked ELP hydrogels showed up to four orders of magnitude greater stiffness than un-cross-linked ELP coacervates and supported chondrocyte growth and cartilaginous matrix synthesis for 28 days.

ELP scaffolds can also be cross-linked prior to implantation. Yee and coworkers (60) used the natural cross-linker genipin to cross-link an ELP to form a stiff hydrogel, which they press-fit into osteochondral defects in rabbits. After 6 weeks, the press-fit gel exhibited an aggregate modulus similar to that of native articular cartilage while still allowing new hyaline cartilage deposition. In a subsequent study (61), they modified an ELP with a thiol-containing hyaluronan and a poly(ethylene glycol) diacrylate cross-linker to produce a material with a higher aggregate modulus (27.6 kPa). This ELP scaffold promoted cell growth in vitro but, due to rapid ELP scaffold degradation in vivo, did not exhibit improved healing in a rabbit disc puncture model. However, improvements to cross-linking density and growth factor addition could be optimized to further enhance its stability.

All these previous studies on ELP-based cross-linked hydrogels utilized monoblock ELPs composed of a single segment. Block copolymers enable control of the microstructure and mechanical properties that go beyond what is possible with ELP monoblock hydrogels (62). ELP block copolymers in a block pattern of ABA or BABA were designed such that the hydrophobic blocks (A) contained THPP–cross-linkable lysine residues, while hydrophilic blocks (B) contained no cross-linking sites. The swelling ratio and gel stiffness of the block copolymer were greater than those of a monoblock polymer lacking the hydrophilic block. Additionally, the mechanical properties were governed by the length of the hydrophilic block, the ratio of block lengths, and the block pattern. The addition of the hydrophilic block enhanced cell viability compared to a monoblock hydrophobic ELP, possibly due to greater porosity and cell infiltration.

Recently, Olsen and coworkers (63) further increased the stiffness of cross-linked ELPs by extending ELP chains by telechelic oxidative coupling, in which cysteine-flanked ELPs are cross-linked continuously in oxidative conditions. Chain extension increases the average molecular weight of the ELP components, resulting in coacervates with more entanglements that promote physical cross-linking and increase gel stiffness. Chain-extended ELPs demonstrated a remarkably greater aggregate modulus—a parameter that describes material stiffness—of up to 1 MPa for a 30 wt% gel while remaining stable and supporting cell growth for over a week.

Fatty Acid–Modified ELPs

While ELPs possess many useful properties for tissue engineering applications, they are limited by the building blocks available to construct them. Recombinant proteins expressed in *E. coli* are composed of 20 canonical amino acids, whereas synthetic polymers consist of far more chemically diverse building blocks (64). Accessing more diverse building blocks—similar to those available for synthetic polymers—has the potential to provide ELPs with a wider variety of structures and properties that may be useful for tissue engineering and other applications.

To create fibrillar structures that mimic the surface topography of the extracellular matrix for tissue engineering applications, Chilkoti and coworkers (65) engineered biohybrid materials called fatty acid–modified ELPs (FAMEs). FAMEs combine a myristoyl group, a β-sheet-forming peptide, and an ELP and are synthesized using one-pot recombinant expression and posttranslational

modification. FAMEs have two segments: The first, N-terminal segment is a β-sheet-forming peptide that also functions as a substrate for *N*-myristoyltransferase (NMT), and the second segment is an ELP. This construct is overexpressed in *E. coli* from a bicistronic plasmid that also expresses yeast NMT. The NMT then myristoylates—adds a C_{14} alkyl chain—covalently to the N terminus of the FAME. The myristoylated peptide segment is a peptide amphiphile that drives hierarchical self-assembly of the FAME, while the ELP segment imparts temperature responsiveness to the hierarchical self-assembly process (65).

FAMEs undergo a three-stage, temperature-dependent hierarchical self-assembly that also depends on the sequence of the β-sheet-forming peptide. At temperatures below the ELP T_t, the FAME forms worm-like micelles composed of a stiff core—driven by the attractive forces of the peptide amphiphiles—and a hydrated ELP corona. Above the T_t, the ELP is dehydrated and the cores aggregate, increasing interactions between nanostructures and forming mesoglobules. In the third stage of assembly, the temperature of the system is raised beyond a second characteristic temperature observed in FAMEs, called the critical temperature. This triggers FAME self-assembly into macroscopic structures ranging from flat sheets to amorphous aggregates to anisotropic fibers (65).

By forming fibrillar structures that mimic the surface topography of the extracellular matrix, FAMEs may provide cells with more natural mechanical and topographical cues. FAMEs have the added benefit of one-pot synthesis, simplifying production compared to that of synthetic peptide amphiphiles (66, 67). Furthermore, because FAMEs are made of a single molecule, biological moieties can be easily fused to FAMEs at the gene level, although how this may impact hierarchical self-assembly needs to be investigated. With further development, FAMEs could provide a highly tunable, genetically controlled alternative to synthetic peptide amphiphiles and other synthetic materials as materials for tissue engineering (65).

Partially Ordered Polymers

ELPs are defined by their complete lack of structural order, and their coacervates and cross-linked hydrogels consequently do not have any internal microstructure. Chilkoti and coworkers (68) sought to study how introducing structural order into disordered ELPs could impact their LCST phase behavior and material properties. To do so, they embedded polyalanine helices, a structural element of tropoelastin, along the ELP sequence. The resulting partially ordered polymers—termed POPs—did not form homogenous coacervates upon thermally triggered phase separation. Instead, they formed physically cross-linked fractal networks with architectures similar to that of cross-linked elastin (68). The porosity and mechanical stability could be tuned by adjusting the polymer concentration and the composition or percentage of helical domains. Interestingly, the POPs remained thermally responsive but demonstrated thermal hysteresis in their phase behavior. The T_t upon heating of an aqueous solution of a POP is largely a function of the ELP sequence and its chain length, but once the solid fractal network that is characteristic of the POP phase transition forms at its T_t, the T_t at which the POP dissolves upon cooling is largely affected by the composition and number of polyalanine domains. Thus, POPs provide a modular system in which various properties can be tuned independently (68).

Like ELPs, the T_t of POPs can be engineered so that POPs can be injected as a liquid and rapidly undergo their phase transition at body temperature. Once injected, POPs assemble into porous, three-dimensional scaffolds with greater stability than that of disordered ELPs. Within one day of subcutaneous injection into a mouse, POPs began integrating into the subcutaneous space. Within 10 days, blood vessels began to form within the POP, and within 3 weeks, more mature and uniform vasculature developed. POP injections resulted in a mild inflammatory response

that quickly dissipated within several days. Within 3 weeks, the number of nonhematopoietic cells surpassed the number of innate immune cells infiltrating the POP network (68). Vascularization and cell proliferation in the POP indicate the promise of this material as a modular tissue engineering scaffold.

Silk ELPs

Although ELPs can form useful architectures for tissue engineering, the poor mechanical properties of ELPs limit their clinical utility (48, 51). To combat this, silk protein derivatives have been combined with ELPs to achieve the high tensile strength of fibroin (the silk protein) and the elasticity of ELPs. These silk–elastin-like polypeptides (SELPs) were created by incorporating the Gly-Ala-Gly-Ala-Gly-Ser amino-acid motif from the *Bombyx mori* silk moth into an ELP sequence. SELPs are recombinantly produced with methods similar to those used to produce ELPs (49, 69). The resulting hybrid material improves the tensile strength of ELPs and the solubility of silk and can assemble into interesting nanostructures for tissue engineering applications (69, 70).

Since they were first developed by Cappello and coworkers (70), SELPs have been extensively studied for tissue engineering applications. One of these applications is cartilage repair and cartilage matrix synthesis. Cappello and coworkers (71) designed an SELP with ELP repeats containing lysine as the guest residue. As the temperature was increased, the SELP underwent an irreversible sol-to-gel transition as hydrogen bonds physically cross-linked the silk domains. The SELP was engineered so that this transition occurred at 37°C, allowing injection in soluble form and gelation at body temperature following injection into the site of the defect, eliminating the need for radiative or chemical cross-linking (71). When human mesenchymal stem cells (hMSCs) were encapsulated in the SELP, their growth and proliferation were supported for 4 weeks. The hMSCs expressed chondrogenic markers, indicating that the SELP provided the proper physiochemical environment for chondrogenesis. Transforming growth factor-β3, a chondrogenic factor, was also added to the culture media, which further enhanced chondrogenic marker expression and collagen accumulation by hMSCs encapsulated in the SELP (71). In another study, SELPs were evaluated as an acellular therapy for cartilage repair. SELPs were injected into osteochondral defects in rabbits and remained physically cross-linked at the site for up to 12 weeks (72). These studies highlight the use of SELPs to promote cartilage matrix synthesis.

More recently, electrospun SELP nanofibers have been used as 3-D scaffolds and skin grafts. An SELP was dissolved in formic acid and electrospun into nanofibers, whose diameter and morphology could be tuned by adjusting the SELP concentration or the number of silk blocks within the copolymer (73). The surface wettability, mechanical properties, and degree of swelling of electrospun ELP nanofiber mats indicated that this material may be suitable for skin regeneration. Indeed, the mats supported fibroblast viability, adhesion, and proliferation. The incorporation of cell recognition sequences may further augment these properties (73).

Further research on SELPs is continuing to evaluate how various factors, including temperature and the silk-to-elastin ratio, affect nanofiber assembly and gelation (74–77). By gaining a better understanding of how SELP morphology and mechanical properties can be precisely tuned, more suitable materials for tissue engineering applications can be designed.

DRUG DELIVERY

ELPs have been widely explored as drug delivery carriers owing to their unique biophysical properties and the use of recombinant DNA methods for design and synthesis (78). ELPs can be conveniently fused with a therapeutic peptide or protein at the gene level and can be site-specifically

conjugated to small molecules using precisely placed residues along the ELP chain that provide one or more unique reactive side chains for covalent conjugation. The composition of the ELP sequence and its molecular weight can be optimized to confer favorable attributes to the therapeutic, including improved pharmacokinetics, lower off-target cytotoxicity, greater stability, and improved solubility. These factors can improve the drug's half-life and bioavailability. One advantage of ELPs as drug carriers is that their degradation by-products are nontoxic, unlike those of many synthetic polymers. Initial work by Chilkoti and coworkers (79, 80) demonstrated that ELP degradation is largely driven by serum elastase and collagenase and occurs over a time frame that is biologically relevant for drug delivery.

For drug delivery applications, ELPs can be engineered to self-assemble into nanoparticles (51, 81) or depots (78, 82) under physiological conditions. For example, in both systems, the ELP length can be optimized to improve delivery of a drug in a tumor by taking advantage of the enhanced permeation and retention effect, the phenomenon by which nanoparticles and macromolecules preferentially accumulate in a tumor owing to the leaky vasculature and poor lymphatic drainage in the tumor (82, 83). Meanwhile, chronic or systemic conditions that require long-term circulation may benefit from ELP depot formation, triggered by ELP LCST behavior, to reduce the frequency of drug administration and maintain a more consistent drug concentration (84). In this section, we focus on ELP-based materials and fusions that have been developed for use as drug delivery carriers.

Soluble ELP Unimers

A limitation of many small-molecule drugs and peptide therapeutics is their short half-life. Despite their potency and, in the case of peptides, specificity, these small therapeutic agents are rapidly cleared from circulation, hindering their efficacy. Frequent dosing is hence required, resulting in dose-dependent side effects. Hydrophobic small-molecule drugs also suffer from poor solubility, restricting their maximum dose and in vivo efficacy (85). Macromolecular carriers such as polyethylene glycol are often used to increase circulation half-life and drug accumulation at the target site, but these formulations face their own toxicity limitations (86). Conjugation of these agents to ELPs, either through recombinant or chemical methods, can be a useful tool for improving the delivery of small-molecule drugs and peptides.

ELPs can also enhance the stability of their fusion partners (87–90) and improve their biodistribution and pharmacokinetic profile compared to free drug. The ELP confers its large size to the attached therapeutic, increasing its serum retention time and slowing renal filtration (91). ELP attachment to a peptide may or may not impact its activity (23, 92), and this must be tested empirically for each application.

Injectable ELP Depots

Thermally responsive ELPs provide a promising drug delivery platform owing to the prolonged presence and tunable solubilization of ELP coacervates in vivo. The ELP T_t can be tuned so that the ELP is soluble at room temperature but forms an insoluble coacervate beneath the skin. The ELP in the coacervate is more dilute at the boundary layer, and as the phase behavior is dependent on ELP concentration (9), the ELP slowly dissolves from its surface to its core to release the therapeutic fusion into the bloodstream (7). Once in circulation, the large ELP impedes renal filtration of the therapeutic (80, 81) (**Figure 6**). By providing sustained release from the injection site and reducing the clearance of the ELP fusion once it is released into systemic circulation, ELP fusions provide two modes to extend the half-life of small molecules and peptides.

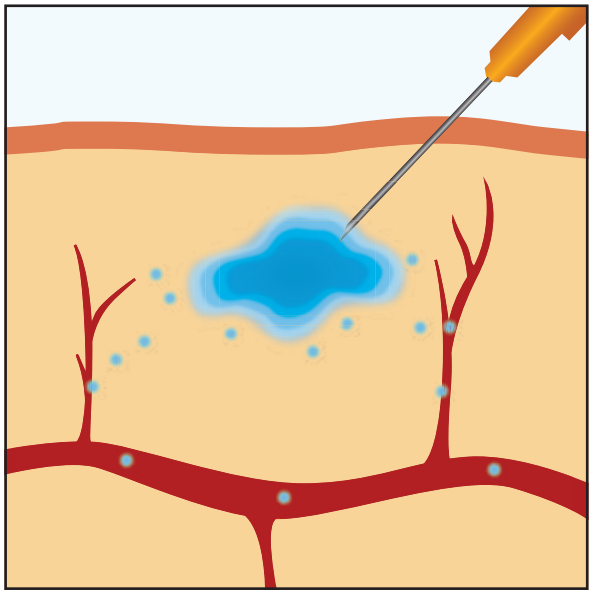

Figure 6

Depot-forming elastin-like polypeptides (ELPs) for drug delivery can be engineered such that their transition temperature (T_t) is between ambient temperature and body temperature, allowing them to be injectable before they form a subcutaneous insoluble coacervate. ELP depots slowly dissolve from their surface to their core, steadily releasing the therapeutic into circulation.

ELP depots have properties that are advantageous for treating diseases that require long-term treatment, such as cancer and chronic illnesses. In one approach, ELP brachytherapy has been explored for in situ radiotherapy of solid tumors. In ELP brachytherapy, an ELP with a T_t below body temperature is chemically conjugated to a radionuclide (^{131}I) to increase retention time of the radionuclide upon injection and to shield the ^{131}I from degradation by dehalogenases. Chilkoti and coworkers (93) demonstrated prolonged localization of the radionuclide in a tumor upon intratumoral injection of a ^{131}I-ELP conjugate that forms a depot in situ because of the phase transition of the conjugate into an insoluble coacervate at a temperature below 37°C. The depot-forming ELP improved drug retention in the tumor by 2.5-fold compared to the soluble ELP fusion and provided more sites for ^{131}I labeling without reducing the T_t below ambient temperature (94). By doubling the ELP chain length, the tumor retention of ^{131}I-ELP was increased by 14-fold compared to the ELP chain with half the molecular weight; by increasing the injection concentration, the drug retention in the tumor was increased by 5-fold compared to injections of the ^{131}I-ELP injected at one-sixteenth the concentration. The optimal dosing concentration, ELP length, and radionuclide labeling resulted in a 100% survival rate in a xenograft FaDu squamous cell model and in a xenograft PC-3 prostate cancer model in nude mice, with over two-thirds of mice showing complete regression.

By cross-linking ELP depots, the rate of degradation can be reduced and drug retention time increased. Initially, cross-linking radionuclide-labeled ELPs involved adding cysteine residues into the ELP backbone to introduce intrachain disulfide cross-linking sites. The addition of 0.3 wt% H_2O_2 reduced gelation time by increasing the oxidation of free thiols to form disulfide bonds, and the gelled depot demonstrated a longer intratumoral retention time in FaDu xenograft mice than was seen in depots that did not undergo H_2O_2-triggered gelation (95). Further work demonstrated

that the incorporation of extrinsic cross-linking sites is unnecessary, as radionuclide ELPs with a hydrophobic $(Tyr\text{-}Gly)_8$ tail—added to provide multiple tyrosine residues for radiolabeling—self-assemble into micelles because of the amphiphilicity of the macromolecule (96, 97). These micelles are subsequently cross-linked by the high-energy β-emissions of the ^{131}I core over 24 h (97). They cross-link into a micellar hydrogel, retain up to 70% of their radioactivity after 60 days, and exhibit significantly lower radioactive accumulation in off-target tissues compared to a soluble radionuclide-labeled unimer.

Sustained drug release also benefits treatment of type 2 diabetes. Many antidiabetic peptides, including insulin and glucagon-like peptide 1 (GLP-1), are limited by their minutes-long half-life and rapid clearance (98, 99). People with diabetes hence require frequent and repeated injections of these peptides to control their blood glucose, a treatment regimen that is inconvenient and uncomfortable (84, 100). A delivery system that prolongs release of these peptides would benefit patients. To address this need, Amiram et al. (101) developed a subcutaneously injected system for sustained release of GLP-1 by fusing GLP-1 with a depot-forming ELP. Fluorescently labeled GLP-1–ELP fusions were tracked in vivo by near-infrared imaging at the subcutaneous injection site, which showed that the depot was retained for up to 5 days after injection. The longer retention time of the GLP-1–ELP fusion compared to GLP-1 translated to a temporally sustained reduction in blood glucose levels in mice: In C57BL/6J mice, a single subcutaneous injection of a depot-forming GLP-1–ELP fusion resulted in lower blood glucose levels for up to 5 days (101). The GLP-1–ELP depot was further optimized by generating a library of fusions to determine the effect of T_t and molecular weight on depot retention and GLP-1 half-life (102). The optimal T_t was approximately 30°C, as depots with a lower T_t resulted in slower release kinetics and less control of blood glucose, whereas depots with a higher T_t showed bolus-type release instead of the desired zero-order release kinetics. ELPs greater than 36 kDa in size showed prolonged circulation times, as their size was greater than the threshold for renal filtration. The fully optimized ELP was retained in the subcutaneous space and exhibited glycemic control for up to 10 days in three different mouse models of diabetes, demonstrating that ELPs can improve the pharmacological properties of small peptides (102).

Another approach to GLP-1 delivery is to introduce protease cleavage sites between the ELP and oligomeric repeats of GLP-1, allowing for sustained release of free GLP-1 (103). Upon subcutaneous injection of such protease-operated depots, proteases in the surrounding environment release GLP-1 from the coacervated depot into circulation. A single subcutaneous injection of a GLP-1 protease-operated depot exhibited controlled blood glucose levels for up to 5 days, 120 times longer than was observed for free GLP-1.

An antidiabetic protein, fibroblast growth factor 21 (FGF21), has also benefited from fusion to an ELP. Gilroy et al. (31) showed that fusion of FGF21 with an ELP improves retention of FGF21 in vivo as well as its expression and purification. FGF21 is typically expressed in inclusion bodies, requiring costly refolding steps prior to downstream purification. The ELP enhances FGF21 solubility, allowing ELP-FGF21 to be expressed in the bacterial cytoplasm and easily purified from cell lysate. The ELP serves as a purification tag through ITC, eliminating the need for chromatography. Importantly, the ELP also allows thermally responsive depot formation for sustained release of the ELP-FGF21 fusion in vivo. Pharmacokinetic studies showed that fusion of an ELP to FGF21 increased its serum half-life 5-fold versus free drug, and fluorescence tomography imaging showed retention of the fusion for up to 4 days following injection. The ELP-FGF21 fusion showed superior weight control and lower blood glucose levels in *ob/ob* mice for up to 5 days, versus 6 h of glucose control for free FGF21. Long-term studies of ELP-FGF21 fusions showed nearly 40% reduction in mean serum insulin and triglyceride levels after 60 days with subcutaneous injections every 5 days (31).

ELP fusions have also been investigated in a preclinical model as a potential treatment for dry eye disease, a chronic ocular surface disease that can result in vision disturbance and instability of tear production (104). Lacritin (Lact) is a 12-kDa protein found in tears that stimulates the lacrimal glands on the corneal epithelium to produce tears. Upon tear production, Lact is rapidly cleared from the ocular surface. Patients with dry eye disease often suffer from a downregulation of Lact production and require constant dosing of hydrating agents. MacKay and coworkers (104) developed a Lact-ELP fusion that coacervates upon injection and retains the presecretory effect of native Lact. While native Lact was undetectable in a nonobese diabetic mouse model of Sjögren's syndrome after 2 h, the Lact-ELP fusion was present even after 48 h.

ELP Nanoparticles

In the past several decades, nanoparticle formulations for improving the delivery and distribution of therapeutic agents have been extensively investigated in preclinical studies (105). Such formulations can prevent premature drug clearance and increase therapeutic efficacy because of their large size and ability to protect a therapeutic payload. Furthermore, the nanoparticle exterior can be decorated with targeting moieties to locate the drug to specific tissues, reducing off-target toxicity. These properties allow for more specific delivery and administration of higher doses to treat a disease more effectively.

The design and production of a nanoparticle can have profound impacts on its in vivo behavior. Size, drug encapsulation efficiency, and biodegradability are all critical considerations in the design of a nanoparticle formulation and can be difficult to control using synthetic polymers. As such, natural polymers have attracted much attention for improving nanoparticle design and production. ELPs are promising candidate materials for nanoparticles owing to their ease of production, thermal responsiveness, and capacity for self-assembly into nanostructures. Self-assembling ELP nanoparticles have been designed to deliver therapeutic agents for a variety of diseases.

ELP block copolymer nanoparticles. ELP amphiphiles consisting of linked hydrophilic and hydrophobic ELP blocks with different T_t values can form micelles for use in drug delivery. At temperatures between their T_t values, the hydrophobic block dehydrates and aggregates to form a micelle core, while the hydrophilic block remains hydrated and forms the micelle corona, remaining exposed to the micelle's surroundings (**Figure 7**). The temperature at which micelle formation occurs is called the critical micellization temperature. This temperature, the micelle diameter, and the number of ELP chains per micelle can all be systematically tuned by altering the ELP sequence and molecular weight and by adjusting the ratio of the two ELP blocks. This design is useful for encapsulating hydrophobic drugs in the micelle core and displaying ligands or targeting moieties on the micelle surface.

Conticello and coworkers (106) were the first to design ELP diblocks that formed micelles with spherical or cylindrical morphologies. They later expanded the ELP block copolymer library to include triblock designs composed of a hydrophilic block flanked by two different hydrophobic blocks. These triblocks formed networks connected via cross-links between hydrophilic domains (107, 108). Chilkoti and coworkers (109) later demonstrated that functional ligands such as Arg-Gly-Asp and Asn-Gly-Arg could be incorporated at the ELP terminus to enhance cell uptake. As a proof of concept, a fluorophore was encapsulated within functionalized ELP nanoparticles with T_t values between 37 and 42°C. At a temperature slightly above body temperature, the ELP micelles were internalized by the cells, a phenomenon that was enhanced by Arg-Gly-Asp and Asn-Gly-Arg targeting (109). This work demonstrated the potential of using targeted ELP nanoparticles for drug delivery.

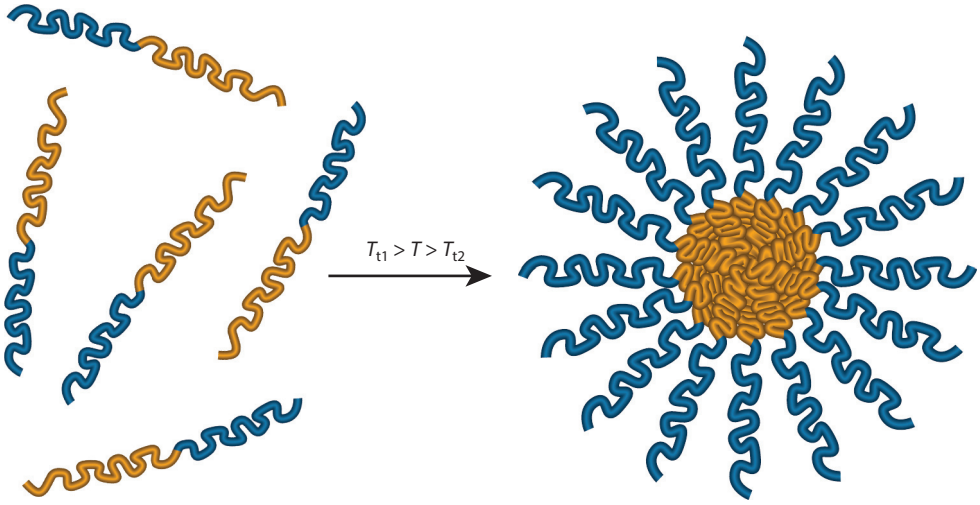

Figure 7

An elastin-like polypeptide (ELP) nanoparticle formed by a diblock ELP. At a temperature between the transition temperature (T_t) of the more hydrophobic ELP block (*orange*, T_{t2}) and the more hydrophilic ELP block (*blue*, T_{t1}), the more hydrophobic block transitions and aggregates while the more hydrophilic block remains soluble, leading to self-assembly into micelles.

Targeted ELP nanoparticles were engineered for tumor-specific drug delivery using a strategy termed dynamic affinity modulation (110). Gly-Arg-Gly-Asp-Ser, a low-affinity ligand used to target the $\alpha_v\beta_3$ integrin, was incorporated at an ELP N terminus, while cysteine residues were added to the ELP C terminus for drug or fluorophore conjugation. The fusion was designed such that at physiological temperature, the ELP was soluble. In this state, Gly-Arg-Gly-Asp-Ser did not interact efficiently with its target integrin. Upon localized hyperthermia, the fusion self-assembled into micelles, increasing the valency and avidity of Gly-Arg-Gly-Asp-Ser and enhancing cell binding and internalization. This strategy mitigated off-target effects of the targeting ligand and reduced systemic toxicity (110).

MacKay and coworkers (111, 112) tested the use of diblock ELPs for the treatment of cancer and autoimmune disease, with rapamycin as the therapeutic agent. The cognate protein target of rapamycin, FK506 binding protein 12 (FKBP12), was fused to the hydrophilic terminus of the ELP diblock. Rapamycin was nonspecifically encapsulated within the nanoparticle core and was also specifically bound to the FKBP12 on the micelle surface (111, 112). Upon administration in vivo, rapamycin in the micelle core was rapidly released into circulation, followed by a more prolonged release of surface-bound rapamycin, resulting in a half-life of 57.8 h (112). When administered in an aggressive breast cancer mouse model, these nanoparticles showed lower off-target toxicity and greater potency, reducing tumor volumes and extending survival time compared to free drug (112). Similar ELP nanoparticles have shown improved outcomes in Sjögren's syndrome, a chronic autoimmune disease characterized by endocrine gland inflammation and lymphocytic infiltration. By increasing rapamycin's half-life and reducing its dose-dependent toxicity, diblock ELPs allowed more effective targeting of the mammalian target of rapamycin pathway and downregulation of proinflammatory cytokines, reducing lymphocytic infiltration into the lacrimal gland (111). These studies demonstrate the potential of using diblock ELP nanoparticles to improve therapeutic potency while reducing toxicity.

More recently, the MacKay group (113, 114) designed a diblock ELP to target liver and lacrimal gland cells. The knob domain of adenovirus serotype 5 fiber protein was fused to an ELP to target coxsackievirus and adenovirus receptor, a cell adhesion protein specifically located on those cell types (113, 114). The ELP nanoparticles were endocytosed by hepatocytes in vitro, indicating that they could be employed as targeted delivery vehicles while avoiding the immunogenicity typically observed with the full adenovirus capsid protein (113). The same nanoparticle was shown to be a potential vehicle for ocular peptide delivery. After intralacrimal injection in mice, the diblock ELP was transcytosed from the basolateral to the apical membrane of lacrimal gland cells while avoiding rapid clearance by tear production (114). These studies show how nanoparticles based on ELP block copolymers can transform targeted drug delivery for more effective and potent treatment.

ELPs have also been combined with protein polymers that exhibit upper critical solution temperature (UCST) behavior to create nanoparticles. Like diblock ELPs, block copolymers with an LCST block and UCST block exhibit self-assembly. Additionally, as temperature increases, the hydrophobicity of the two blocks is swapped. The UCST block, which aggregates in the nanoparticle core at low temperatures, becomes more hydrated as the ELP coacervates (115, 116). LCST-UCST copolymers were previously shown to form spherical or vesicle morphologies, but the design principles that drive their assembly were unclear (117, 118). To elucidate the parameters that control self-assembly, Chilkoti and coworkers (119) designed a diblock copolymer with an ELP and resilin-like polypeptide (RLP), a protein polymer composed of repeating monomers of the octapeptide Gln-Tyr-Pro-Ser-Asp-Gly-Arg-Gly that demonstrates UCST behavior. They discovered that two key factors—the RLP:ELP ratio and ELP hydrophilicity—strongly impacted nanoparticle formation and morphology. As the RLP:ELP ratio increased, the aggregation number and nanoparticle radius also increased. Meanwhile, nanoparticle morphology could be tuned by altering ELP hydrophilicity. A hydrophobic ELP promoted the formation of spherical nanoparticles, but when it was swapped with a hydrophilic ELP, the copolymer formed worm-like micelles (119).

The tunable morphology of RLP-ELP copolymers was instrumental to the presentation of targeting moieties on the nanoparticle surface. Chilkoti and coworkers (120) incorporated an $\alpha_v\beta_3$ integrin-targeting fibronectin type III (Fn3) onto the C terminus of the RLP-ELP such that it was displayed on the nanoparticle's ELP corona without perturbing self-assembly. They discovered that the tunable morphology and high avidity of nanoparticles of RLP-ELP-Fn3 dramatically improved binding kinetics to the $\alpha_v\beta_3$ integrin. Compared to an RLP-ELP-Fn3 control that does not form nanoparticles, Fn3-decorated spherical micelles demonstrated a 10-fold increase in avidity. Meanwhile, Fn3-decorated worm-like micelles had a 1,000-fold increased avidity, driving the equilibrium dissociation constant down to a picomolar concentration (120). It is believed that, by extending the sphere into a flexible, worm-like micelle, the nanoparticle can interact with and bind to a greater number of receptors. The cylindrical morphology allows the nanoparticle to take advantage of its multivalency more effectively, with its affinity surpassing that of a clinically relevant therapeutic antibody with a dissociation constant of 20 nM (120). Indeed, when cells expressing high levels of the $\alpha_v\beta_3$ integrin were treated with the antibody or the Fn3-decorated micelles, the worm-like micelles demonstrated higher cellular uptake than the antibody did. This robust targeting strategy, coupled with the high thermodynamic stability of these micelles, could provide a versatile system for the delivery of drugs or imaging agents (120).

ELP hybrid nanoparticles. ELP hybrid materials consisting of an ELP fused to other polymers, proteins, or molecules can be designed to assemble into nanoparticles. Like ELP block copolymers, nanoparticle assembly is driven largely by hydrophobic interactions.

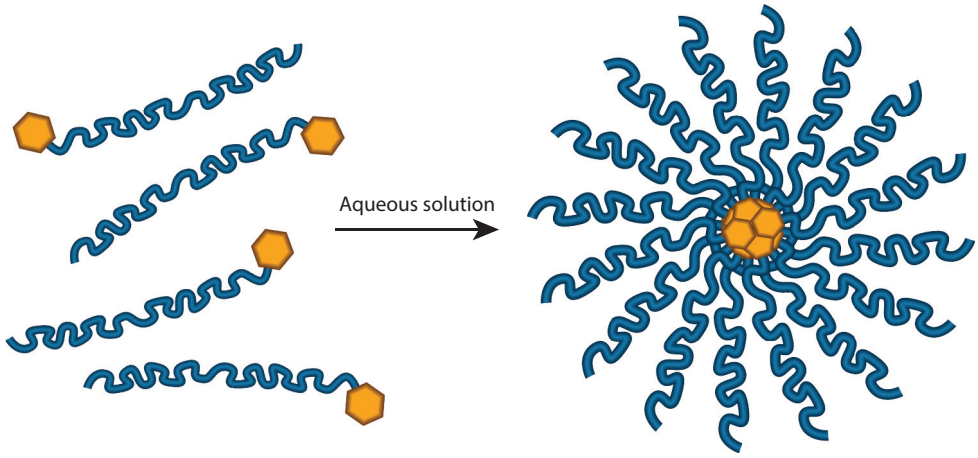

Figure 8

Attachment-directed assembly of micelles (ADAM). In ADAM, one or more copies of a hydrophobic cargo (*orange*) are chemically attached to one end of an elastin-like polypeptide (ELP) (*blue*), which triggers its self-assembly into micelles in an aqueous environment, with the cargo sequestered in the core of the micelle.

Chemical conjugation of small molecules to ELPs can drive the formation of nanoparticles through a bottom-up nanoparticle fabrication method that Chilkoti and coworkers (121) named attachment-directed assembly of micelles (ADAM). In ADAM, attaching multiple copies of a small hydrophobic molecule with a logD $\geq$ 1.5 to one end of an unstructured hydrophilic polypeptide triggers its self-assembly into <100-nm micelles in an aqueous environment, sequestering the small molecule in the core of the micelle (121) (**Figure 8**). MacKay et al. (122) demonstrated the utility of ADAM by conjugating multiple copies of doxorubicin—a chemotherapeutic—to a hydrophilic ELP via a pH-sensitive linker. This ELP, which had a T_t above body temperature, was recombinantly fused to a cysteine-rich segment that was used as the drug attachment site, and this fusion was named a chimeric polypeptide (CP). At concentrations above 3 μM, CP-doxorubicin nanoparticles self-assembled into nanoparticles with a hydrodynamic radius of 21 nm. The CP-doxorubicin nanoparticles remained stable at physiological pH but rapidly released doxorubicin at pH 5, a pH seen in late endosomes and lysosomes (122). Doxorubicin conjugation resulted in a 4-fold greater maximum tolerated dose versus free drug, as the CP micelle reduced off-target toxicity. With only a single injection, CP-doxorubicin micelles reduced tumor size nearly 13-fold compared to doxorubicin alone and cured eight of nine mice after 2 months (122). A later study showed that CP-doxorubicin enhanced antitumor immunity, likely due to increased accumulation of the drug in the tumor (123).

Bhattacharyya et al. (124) further investigated ADAM for drug delivery using the chemotherapeutic paclitaxel. Paclitaxel was covalently conjugated to the CP via an acid-cleavable linker, and the conjugate self-assembled into nanoparticles with a hydrodynamic radius of 33 nm. Like CP-doxorubicin, these CP-paclitaxel nanoparticles released their drug payload more efficiently at an acidic pH—one that enables drug release in the endolysosomes of cells—due to conjugation via an acid-labile linker. CP-paclitaxel significantly outperformed Abraxane, an albumin-binding formulation of paclitaxel, in breast and prostate cancer models. Notably, off-target accumulation of paclitaxel decreased 27-fold when the drug was encapsulated in the nanoparticle (124). Bhattacharyya and coworkers (125) also designed a CP-niclosamide nanoparticle for treatment of colorectal cancer. These micelles exhibited a hydrodynamic radius of 30 nm and, like CP-doxorubicin and

CP-paclitaxel, demonstrated rapid drug release in acidic conditions. Interestingly, CP-niclosamide formed rod-like micelles rather than spherical structures. The average survival time increased from 16 days for treatment with free niclosamide to 26 days for mice treated with the same drug-equivalent dose of CP-niclosamide. In addition, CP-niclosamide demonstrated a reduction in β-catenin signaling that was similar to that of free drug (125). Collectively, these studies highlight the advantages of using ELP–drug hybrid nanoparticles to improve the efficacy, dosing, and distribution of small-molecule drugs.

The original implementation of ADAM only works when the small molecule conjugated to the CP has a logD $\geq$ 1.5. Although many cancer drugs are hydrophobic, a significant number of drugs are hydrophilic. To expand the scope of ADAM to hydrophilic drugs, Chilkoti and coworkers (126) built on the findings of a study (96) in which they demonstrated the spontaneous self-assembly of ELPs into cylindrical micelles by fusing a short, 1.5-kDa $(XG_y)_8$ assembly domain to one end of the ELP where the residue X is phenylalanine, tyrosine, or tryptophan and *y* ranges from 0 to 2. Building on this work, they designed a recombinant asymmetric triblock polypeptide that consists of three segments: an ELP, a hydrophobic tyrosine-rich segment, and a short cysteine-rich segment (126). Covalent conjugation of a structurally diverse set of hydrophilic small molecules, including a hydrophilic chemotherapeutic—gemcitabine—to the cysteine residues leads to formation of rod-shaped micelles. Gemcitabine-loaded asymmetric triblock polypeptide micelles showed significantly better tumor regression compared to free drug in a preclinical mouse cancer model. This approach allows conjugation-driven encapsulation of diverse, hydrophilic, small-molecule drugs and imaging agents to a suitable reactive group.

In the examples above, drug attachment is achieved by chemical conjugation to a specific, naturally occurring amino acid in the ELP sequence. Unfortunately, these resides can also be promiscuously distributed in targeting moieties that are recombinantly added to the nanoparticle surface, hindering site-specific attachment of the drug. Unnatural amino acids can also provide a unique reactive site for site-specific drug conjugation. Chilkoti and coworkers (127) incorporated the unnatural amino acid *p*-acetylphenylalanine at the N terminus of an ELP as a bioorthogonal reactive moiety. The *p*-acetylphenylalanine contained a ketone group to which doxorubicin could be attached via a pH-sensitive linker. An EgA1 nanobody was incorporated at the C terminus to target the epidermal growth factor receptor. This nanoparticle showed enhanced drug uptake and potency in two epidermal growth factor receptor–positive cancer cell lines, demonstrating the utility of an ELP nanoparticle to enable site-specific drug conjugation and tumor cell targeting (127).

SELPs have also been employed as delivery vehicles (128, 129). SELP nanoparticle formation is spontaneous and driven by hydrogen bonding between silk domains. Nanoparticle properties can be controlled by tuning the silk:ELP block ratio and by altering the ELP T_t through choice of guest residue (74). In 2014, Xia et al. (130) demonstrated that SELP nanoparticles could be used for tumor drug delivery. Doxorubicin was mixed with SELP fusions and became entrapped in the SELP core owing to hydrophobic interactions. By tuning the silk:ELP block ratio, they obtained nanoparticles with mean hydrodynamic radii ranging from 50 to 142 nm. When delivered to HeLa cells in vitro, the nanoparticles showed 1.8-fold greater cytotoxicity than free drug. Confocal microscopy results suggested that the SELP nanoparticle facilitates drug uptake via endocytosis rather than via membrane diffusion, allowing the therapeutic to accumulate in the cytoplasm and diffuse slowly to the nucleus (130).

Fatty acids can also be used to trigger ELP nanoparticle assembly for use in drug delivery. Luginbuhl et al. (131) added a myristoyl group to an ELP during posttranslational modification by using NMT and adding exogenous myristic acid to the culture medium. The resulting myristoylated ELPs (M-ELPs) self-assembled into rod-like or spherical micelles depending on the size of the ELP block. When stirred overnight with dissolved doxorubicin or paclitaxel, M-ELPs

entrapped the chemotherapeutics through hydrophobic interactions with the myristoyl group. This method captured more drug with greater encapsulation efficiency than traditional diblock ELPs (131). The nanoparticles were endocytosed by 4T1 cells in a preliminary pharmacokinetic study, and the M-ELPs increased the drug's half-life by 6.5-fold while reducing the free drug concentration in circulation (131). M-ELPs thus appear promising for entrapping hydrophobic drugs that lose potency upon conjugation or that have no available conjugation strategy.

ELP block copolymers and hybrid materials provide numerous useful platforms by which chemotherapeutic agents, immunosuppressants, and other drugs can be delivered to specific cell types. ELP block copolymers and hybrid materials can be engineered to target diseased tissue and mitigate systemic toxicity and to incorporate domains for site-specific drug conjugation. The precise control over targeting, drug attachment, and nanoparticle properties makes ELPs promising as drug delivery vehicles for a broad variety of diseases.

Zwitterionic Polypeptides

Compared to synthetic drug carriers, ELPs benefit from monodispersity, controlled biological properties, and lower immunogenicity. However, ELPs lack the stealth properties of some synthetic polymers, such as polyethylene glycol and zwitterionic polymers (132–134). Stealth behavior protects the payload from opsonization and premature clearance from circulation, resulting in a better pharmacokinetic profile (135, 136). If ELPs could impart this property to their payload, they would be a promising alternative to current state-of-the-art drug carriers.

To achieve stealth behavior in biopolymers, Banskota and colleagues (137) designed zwitterionic polypeptides (ZIPPs) based on the intrinsically disordered ELP scaffold. ZIPPs were composed of repeats of the Val-Pro-X_1-X_2-Gly motif, where X_1 and X_2 are cationic and anionic amino acids, respectively. The choice of a cationic-anionic residue pair was inspired by synthetic zwitterionic polymers that have been shown to have stealth behavior (138, 139). Like ELPs, ZIPPs can be expressed in *E. coli*, purified without chromatography, and recombinantly fused to a therapeutic peptide. Unlike ELPs, ZIPPs impart high solubility by nature of their charged amino acids, extending the half-life of their therapeutic payload. Compared to an uncharged ELP control, a ZIPP demonstrated 3-fold greater half-life and 2–3-fold greater bioavailability (137). When fused to GLP-1, this ZIPP exhibited 1.5-fold longer blood glucose control than did an uncharged ELP fusion. These studies indicate that ZIPPs possess the favorable properties of ELPs (easy and large-scale purification, monodispersity, easy fusion to therapeutic peptides, and sequence-level control of properties) with stealth behavior previously attainable only by the use of synthetic stealth polymers. ZIPPs may serve as a novel drug delivery carrier to increase the therapeutic efficacy and circulation time of therapeutic cargo (137).

CONCLUSION

Remarkable advances have been made to develop ELPs into versatile, customizable biomaterials for a variety of biomedical applications. Because they offer such exquisite control over their sequence, there are many opportunities to tune their mechanical, chemical, and biological properties. By tuning the thermally responsive behavior of ELPs or attaching bioactive groups, these polypeptides can be engineered for a variety of tissue engineering and drug delivery applications. More recently, groundbreaking research on ELP-based materials has introduced new architectures that can be harnessed to address the needs of the field.

The recombinant nature of ELPs provides much opportunity for the synthesis of diverse macromolecules with unique material properties. Although some research has made progress

in exploring new ELP architectures and biofunctionality, further research on ELPs with new—unnatural amino *cis*—and posttranslational modifications will produce novel ELPs that improve the mechanical properties of the material or provide additional bioorthogonal ligation sites to the molecule. With these new chemistries, more bioactive small molecules or peptides may be introduced to the molecule, making ELPs a more versatile platform for regenerative medicine or drug delivery. Additionally, exploring novel ELP-based sequences beyond the traditional Val-Pro-Gly-X-Gly pentapeptide motif could offer another strategy for developing new ELP-based materials. This tactic was demonstrated by Banskota and coworkers (137) to improve stealth behavior through the design of the ZIPP library; further work to discover similar molecules may provide opportunities to improve the biological behavior of ELPs.

Currently, most published work on ELPs employs bacterial expression systems—typically *E. coli*—to produce the polypeptide. The appeal of this expression system is clear: bacteria rapidly produce high yields of the polypeptide. However, bacterial expression systems restrict the use of ELP fusion partners to peptides and proteins that do not require glycosylation and can properly fold in the bacterial cytoplasm (17). A mammalian expression system, which would contain the necessary enzymes and chaperones to facilitate appropriate posttranslational modification and folding, would make it possible to append a greater number of more diverse proteins to the ELP (140). Additionally, bacterial cell walls contain high levels of inflammatory agents, including endotoxin, which must be removed before ELP fusions are administered in a drug delivery or tissue engineering context. This concern would be eliminated with mammalian expression systems.

In summary, with their unique physicochemical behavior and high level of customizability, ELPs and their derivatives will continue to provide an attractive platform for a variety of biomedical applications. These biopolymers possess many interesting features, including their LCST behavior, biocompatibility, and ability to display bioactive moieties. They are—and will continue to be—a useful material in tissue engineering and drug delivery applications.

DISCLOSURE STATEMENT

A.C. is the inventor of ELP technology for drug delivery that has been licensed by Duke University to PhaseBio Pharmaceuticals, a company he cofounded that is commercializing this technology for the delivery of peptide and protein drugs.

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LITERATURE CITED

1. Abascal NC, Regan L. 2018. The past, present and future of protein-based materials. *Open Biol*. 8:180113
2. Yang YJ, Holmberg AL, Olsen BD. 2017. Artificially engineered protein polymers. *Annu. Rev. Chem. Biomol. Eng.* 8:549–75
3. Haider M, Leung V, Ferrari F, Crissman J, Powell J, et al. 2005. Molecular engineering of silk-elastinlike polymers for matrix-mediated gene delivery: biosynthesis and characterization. *Mol. Pharm.* 2:139–50
4. Mithieux SM, Weiss AS. 2005. Elastin. *Adv. Protein Chem.* 70:437–61
5. Almine JF, Bax DV, Mithieux SM, Nivison-Smith L, Rnjak J, et al. 2010. Elastin-based materials. *Chem. Soc. Rev.* 39:3371–79
6. Wise SG, Yeo GC, Hiob MA, Rnjak-Kovacina J, Kaplan DL, et al. 2014. Tropoelastin: a versatile, bioactive assembly module. *Acta Biomater*. 10:1532–41

7. MacEwan SR, Chilkoti A. 2010. Elastin-like polypeptides: biomedical applications of tunable biopolymers. *Pept. Sci.* 94:60–77
8. Zhao B, Li NK, Yingling YG, Hall CK. 2016. LCST behavior is manifested in a single molecule: elastin-like polypeptide $(VPGVG)_n$. *Biomacromolecules* 17:111–18
9. Meyer DE, Chilkoti A. 2004. Quantification of the effects of chain length and concentration on the thermal behavior of elastin-like polypeptides. *Biomacromolecules* 5:846–51
10. Chilkoti A, Christensen T, MacKay JA. 2006. Stimulus responsive elastin biopolymers: applications in medicine and biotechnology. *Curr. Opin. Chem. Biol.* 10:652–57
11. Hickey JW, Santos JL, Williford J-M, Mao H-Q. 2015. Control of polymeric nanoparticle size to improve therapeutic delivery. *J. Control. Release* 219:536–47
12. Simone EA, Dziubla TD, Muzykantov VR. 2008. Polymeric carriers: role of geometry in drug delivery. *Expert Opin. Drug Deliv.* 5:1283–300
13. Trabbic-Carlson K, Meyer DE, Liu L, Piervincenzi R, Nath N, et al. 2004. Effect of protein fusion on the transition temperature of an environmentally responsive elastin-like polypeptide: a role for surface hydrophobicity? *Protein Eng. Des. Sel.* 17:57–66
14. Christensen T, Hassouneh W, Trabbic-Carlson K, Chilkoti A. 2013. Predicting transition temperatures of elastin-like polypeptide fusion proteins. *Biomacromolecules* 14:1514–19
15. Nair LS, Laurencin CT. 2007. Biodegradable polymers as biomaterials. *Prog. Polym. Sci.* 32:762–98
16. Amiram M, Garcia Quiroz F, Callahan DJ, Chilkoti A. 2011. A highly parallel method for synthesizing DNA repeats enables the discovery of 'smart' protein polymers. *Nat. Mater.* 10:141–48
17. Rosano GL, Ceccarelli EA. 2014. Recombinant protein expression in *Escherichia coli*: advances and challenges. *Front. Microbiol.* 5:172
18. Tang NC, Chilkoti A. 2016. Combinatorial codon scrambling enables scalable gene synthesis and amplification of repetitive proteins. *Nat. Mater.* 15:419–24
19. White MJ, Fristensky BW, Falconet D, Childs LC, Watson JC, et al. 1992. Expression of the chlorophyll-a/b-protein multigene family in pea (*Pisum sativum* L.). *Planta* 188:190–98
20. McMillan RA, Conticello VP. 2000. Synthesis and characterization of elastin-mimetic protein gels derived from a well-defined polypeptide precursor. *Macromolecules* 33:4809–21
21. McMillan RA, Lee TAT, Conticello VP. 1999. Rapid assembly of synthetic genes encoding protein polymers. *Macromolecules* 32:3643–48
22. Meyer DE, Chilkoti A. 2002. Genetically encoded synthesis of protein-based polymers with precisely specified molecular weight and sequence by recursive directional ligation: examples from the elastin-like polypeptide system. *Biomacromolecules* 3:357–67
23. Christensen T, Amiram M, Dagher S, Trabbic-Carlson K, Shamji MF, et al. 2009. Fusion order controls expression level and activity of elastin-like polypeptide fusion proteins. *Protein Sci.* 18:1377–87
24. McDaniel JR, Mackay JA, Garcia Quiroz F, Chilkoti A. 2010. Recursive directional ligation by plasmid reconstruction allows rapid and seamless cloning of oligomeric genes. *Biomacromolecules* 11:944–52
25. Meyer DE, Chilkoti A. 1999. Purification of recombinant proteins by fusion with thermally-responsive polypeptides. *Nat. Biotechnol.* 17:1112–15
26. Christensen T, Trabbic-Carlson K, Liu W, Chilkoti A. 2007. Purification of recombinant proteins from *Escherichia coli* at low expression levels by inverse transition cycling. *Anal. Biochem.* 360:166–68
27. Banki MR, Feng L, Wood DW. 2005. Simple bioseparations using self-cleaving elastin-like polypeptide tags. *Nat. Methods* 2:659–62
28. Shen Y, Ai H-X, Song R, Liang Z-N, Li J-F, Zhang S-Q. 2010. Expression and purification of moricin CM4 and human β-defensins 4 in *Escherichia coli* using a new technology. *Microbiol. Res.* 165:713–18
29. Trabbic-Carlson K, Liu L, Kim B, Chilkoti A. 2004. Expression and purification of recombinant proteins from *Escherichia coli*: comparison of an elastin-like polypeptide fusion with an oligohistidine fusion. *Protein Sci.* 13:3274–84
30. Hassouneh W, MacEwan SR, Chilkoti A. 2012. Fusions of elastin-like polypeptides to pharmaceutical proteins. *Methods Enzymol.* 502:215–37
31. Gilroy CA, Roberts S, Chilkoti A. 2018. Fusion of fibroblast growth factor 21 to a thermally responsive biopolymer forms an injectable depot with sustained anti-diabetic action. *J. Control. Release* 277:154–64

32. Hassouneh W, Christensen T, Chilkoti A. 2010. Elastin-like polypeptides as a purification tag for recombinant proteins. *Curr. Protoc. Protein Sci.* 61:6.11.1–6.11.16
33. Ge X, Yang DSC, Trabbic-Carlson K, Kim B, Chilkoti A, Filipe CDM. 2005. Self-cleavable stimulus responsive tags for protein purification without chromatography. *J. Am. Chem. Soc.* 127:11228–29
34. Wu W-Y, Mee C, Califano F, Banki R, Wood DW. 2006. Recombinant protein purification by self-cleaving aggregation tag. *Nat. Protoc.* 1:2257
35. Bellucci JJ, Amiram M, Bhattacharyya J, McCafferty D, Chilkoti A. 2013. Three-in-one chromatography-free purification, tag removal, and site-specific modification of recombinant fusion proteins using sortase A and elastin-like polypeptides. *Angew. Chem. Int. Ed.* 52:3703–8
36. Bellucci JJ, Bhattacharyya J, Chilkoti A. 2015. A noncanonical function of sortase enables site-specific conjugation of small molecules to lysine residues in proteins. *Angew. Chem. Int. Ed.* 54:441–45
37. Qi Y, Amiram M, Gao W, McCafferty DG, Chilkoti A. 2013. Sortase-catalyzed initiator attachment enables high yield growth of a stealth polymer from the C terminus of a protein. *Macromol. Rapid Commun.* 34:1256–60
38. Gräslund S, Nordlund P, Weigelt J, Hallberg M, Bray J, et al. 2008. Protein production and purification. *Nat. Methods* 5:135–46
39. O'Brien FJ. 2011. Biomaterials & scaffolds for tissue engineering. *Mater. Today* 14:88–95
40. Khan F, Tanaka M. 2017. Designing smart biomaterials for tissue engineering. *Int. J. Mol. Sci.* 19:17
41. Dhandayuthapani B, Yoshida Y, Maekawa T, Kumar DS. 2011. Polymeric scaffolds in tissue engineering application: a review. *Int. J. Polym. Sci.* 2011:290602
42. Petsch D, Anspach FB. 2000. Endotoxin removal from protein solutions. *J. Biotechnol.* 76:97–119
43. Urry DW, Haynes B, Harris RD. 1986. Temperature dependence of length of elastin and its polypentapeptide. *Biochem. Biophys. Res. Commun.* 141:749–55
44. Zhang Q, Weber C, Schubert US, Hoogenboom R. 2017. Thermoresponsive polymers with lower critical solution temperature: from fundamental aspects and measuring techniques to recommended turbidimetry conditions. *Mater. Horiz.* 4:109–16
45. Li NK, Garcia Quiroz F, Hall CK, Chilkoti A, Yingling YG. 2014. Molecular description of the LCST behavior of an elastin-like polypeptide. *Biomacromolecules* 15:3522–30
46. Kammer H-W. 1989. Upper and lower critical solution temperature behaviour in polymer blends and its thermodynamic interpretation. *Polymer* 30:888–92
47. McDaniel JR, Radford DC, Chilkoti A. 2013. A unified model for de novo design of elastin-like polypeptides with tunable inverse transition temperatures. *Biomacromolecules* 14:2866–72
48. Nettles DL, Chilkoti A, Setton LA. 2010. Applications of elastin-like polypeptides in tissue engineering. *Adv. Drug Deliv. Rev.* 62:1479–85
49. Coenen AMJ, Bernaerts KV, Harings JAW, Jockenhoevel S, Ghazanfari S. 2018. Elastic materials for tissue engineering applications: natural, synthetic, and hybrid polymers. *Acta Biomater.* 79:60–82
50. McHale MK, Setton LA, Chilkoti A. 2005. Synthesis and in vitro evaluation of enzymatically cross-linked elastin-like polypeptide gels for cartilaginous tissue repair. *Tissue Eng.* 11:1768–79
51. Le DHT, Sugawara-Narutaki A. 2019. Elastin-like polypeptides as building motifs toward designing functional nanobiomaterials. *Mol. Syst. Des. Eng.* 4:545–65
52. Betre H, Setton LA, Meyer DE, Chilkoti A. 2002. Characterization of a genetically engineered elastin-like polypeptide for cartilaginous tissue repair. *Biomacromolecules* 3:910–16
53. Betre H, Ong SR, Guilak F, Chilkoti A, Fermor B, Setton LA. 2006. Chondrocytic differentiation of human adipose-derived adult stem cells in elastin-like polypeptide. *Biomaterials* 27:91–99
54. Betre H, Chilkoti A, Setton LA. 2002. A two-step chondrocyte recovery system based on thermally sensitive elastin-like polypeptide scaffolds for cartilage tissue engineering. In *Proceedings of the Second Joint 24th Annual Conference and the Annual Fall Meeting of the Biomedical Engineering Society*, pp. 829–30. New York: IEEE
55. Urry DW, Haynes B, Zhang H, Harris RD, Prasad KU. 1988. Mechanochemical coupling in synthetic polypeptides by modulation of an inverse temperature transition. *PNAS* 85:3407–11
56. Nicol A, Gowda DC, Urry DW. 1992. Cell adhesion and growth on synthetic elastomeric matrices containing ARG-GLY–ASP–SER–^{3}. *J. Biomed. Mater. Res.* 26:393–413

57. Trabbic-Carlson K, Setton LA, Chilkoti A. 2003. Swelling and mechanical behaviors of chemically cross-linked hydrogels of elastin-like polypeptides. *Biomacromolecules* 4:572–80
58. Lim DW, Nettles DL, Setton LA, Chilkoti A. 2007. Rapid cross-linking of elastin-like polypeptides with (hydroxymethyl)phosphines in aqueous solution. *Biomacromolecules* 8:1463–70
59. Nettles DL, Kitaoka K, Hanson NA, Flahiff CM, Mata BA, et al. 2008. In situ crosslinking elastin-like polypeptide gels for application to articular cartilage repair in a goat osteochondral defect model. *Tissue Eng. Part A* 14:1133–40
60. Hrabchak C, Rouleau J, Moss I, Woodhouse K, Akens M, et al. 2010. Assessment of biocompatibility and initial evaluation of genipin cross-linked elastin-like polypeptides in the treatment of an osteochondral knee defect in rabbits. *Acta Biomater*. 6:2108–15
61. Moss IL, Gordon L, Woodhouse KA, Whyne C, Yee AJ. 2008. *Nucleus pulposus tissue repair in intervertebral disc degeneration: biochemical and mechanical evaluation of a novel human disc cell – hyaluronan/elastin polypeptide scaffold composite*. Poster 1495 presented at the 54th Annual Meeting of the Orthopaedic Research Society, San Francisco
62. Lim DW, Nettles DL, Setton LA, Chilkoti A. 2008. In situ cross-linking of elastin-like polypeptide block copolymers for tissue repair. *Biomacromolecules* 9:222–30
63. Glassman MJ, Avery RK, Khademhosseini A, Olsen BD. 2016. Toughening of thermoresponsive arrested networks of elastin-like polypeptides to engineer cytocompatible tissue scaffolds. *Biomacromolecules* 17:415–26
64. Hochkoeppler A. 2013. Expanding the landscape of recombinant protein production in *Escherichia coli*. *Biotechnol. Lett*. 35:1971–81
65. Mozhdehi D, Luginbuhl KM, Simon JR, Dzuricky M, Berger R, et al. 2018. Genetically encoded lipid–polypeptide hybrid biomaterials that exhibit temperature-triggered hierarchical self-assembly. *Nat. Chem.* 10:496–505
66. Hendricks MP, Sato K, Palmer LC, Stupp SI. 2017. Supramolecular assembly of peptide amphiphiles. *Acc. Chem. Res.* 50:2440–48
67. Qiu F, Chen Y, Tang C, Zhao X. 2018. Amphiphilic peptides as novel nanomaterials: design, self-assembly and application. *Int. J. Nanomed.* 13:5003–22
68. Roberts S, Harmon TS, Schaal JL, Miao V, Li K, et al. 2018. Injectable tissue integrating networks from recombinant polypeptides with tunable order. *Nat. Mater.* 17:1154–63
69. Roberts EG, Rim N-G, Huang W, Tarakanova A, Yeo J, et al. 2018. Fabrication and characterization of recombinant silk-elastin-like-protein (SELP) fiber. *Macromol. Biosci.* 18:1800265
70. Cappello J, Crissman J, Dorman M, Mikolajczak M, Textor G, et al. 1990. Genetic engineering of structural protein polymers. *Biotechnol. Prog.* 6:198–202
71. Haider M, Cappello J, Ghandehari H, Leong KW. 2008. In vitro chondrogenesis of mesenchymal stem cells in recombinant silk-elastinlike hydrogels. *Pharm. Res.* 25:692–99
72. Nettles DL, Vail TP, Flahiff CM, Walkenhorst J, Carter AJ, Setton LA. 2005. *Injectable silk-elastin for articular cartilage defect repair*. Poster presented at the 51st Annual Meeting of the Orthopaedic Research Society, Washington, DC
73. Machado R, da Costa A, Sencadas V, Garcia-Arévalo C, Costa CM, et al. 2013. Electrospun silk-elastin-like fibre mats for tissue engineering applications. *Biomed. Mater.* 8:065009
74. Xia X-X, Xu Q, Hu X, Qin G, Kaplan DL. 2011. Tunable self-assembly of genetically engineered silk–elastin-like protein polymers. *Biomacromolecules* 12:3844–50
75. Willems L, Roberts S, Weitzhandler I, Chilkoti A, Mastrobattista E, et al. 2019. Inducible fibril formation of silk–elastin diblocks. *ACS Omega* 4:9135–43
76. Zeng L, Jiang L, Teng W, Cappello J, Zohar Y, Wu X. 2014. Engineering aqueous fiber assembly into silk-elastin-like protein polymers. *Macromol. Rapid Commun.* 35:1273–79
77. Johnson S, Ko YK, Varongchayakul N, Lee S, Cappello J, et al. 2012. Directed patterning of the self-assembled silk-elastin-like nanofibers using a nanomechanical stimulus. *Chem. Commun.* 48:10654–56
78. MacEwan SR, Chilkoti A. 2014. Applications of elastin-like polypeptides in drug delivery. *J. Control. Release* 190:314–30

79. Liu W, Dreher MR, Chow DC, Zalutsky MR, Chilkoti A. 2006. Tracking the in vivo fate of recombinant polypeptides by isotopic labeling. *J. Control. Release* 114:184–92
80. Liu W, Dreher MR, Furgeson DY, Peixoto KV, Yuan H, et al. 2006. Tumor accumulation, degradation and pharmacokinetics of elastin-like polypeptides in nude mice. *J. Control. Release* 116:170–78
81. Despanie J, Dhandhukia JP, Hamm-Alvarez SF, MacKay JA. 2016. Elastin-like polypeptides: therapeutic applications for an emerging class of nanomedicines. *J. Control. Release* 240:93–108
82. McDaniel JR, Callahan DJ, Chilkoti A. 2010. Drug delivery to solid tumors by elastin-like polypeptides. *Adv. Drug Deliv. Rev.* 62:1456–67
83. Saxena R, Nanjan MJ. 2015. Elastin-like polypeptides and their applications in anticancer drug delivery systems: a review. *Drug Deliv.* 22:156–67
84. Gilroy CA, Luginbuhl KM, Chilkoti A. 2016. Controlled release of biologics for the treatment of type 2 diabetes. *J. Control. Release* 240:151–64
85. Booth JJ, Abbott S, Shimizu S. 2012. Mechanism of hydrophobic drug solubilization by small molecule hydrotropes. *J. Phys. Chem. B* 116:14915–21
86. Veronese FM, Mero A. 2008. The impact of PEGylation on biological therapies. *BioDrugs* 22:315–29
87. Patel J, Zhu H, Menassa R, Gyenis L, Richman A, Brandle J. 2007. Elastin-like polypeptide fusions enhance the accumulation of recombinant proteins in tobacco leaves. *Transgenic Res.* 16:239–49
88. Kaldis A, Ahmad A, Reid A, McGarvey B, Brandle J, et al. 2013. High-level production of human interleukin-10 fusions in tobacco cell suspension cultures. *Plant Biotechnol. J.* 11:535–45
89. Conley AJ, Joensuu JJ, Jevnikar AM, Menassa R, Brandle JE. 2009. Optimization of elastin-like polypeptide fusions for expression and purification of recombinant proteins in plants. *Biotechnol. Bioeng.* 103:562–73
90. Floss DM, Sack M, Arcalis E, Stadlmann J, Quendler H, et al. 2009. Influence of elastin-like peptide fusions on the quantity and quality of a tobacco-derived human immunodeficiency virus-neutralizing antibody. *Plant Biotechnol. J.* 7:899–913
91. Conrad U, Plagmann I, Malchow S, Sack M, Floss DM, et al. 2011. ELPylated anti-human TNF therapeutic single-domain antibodies for prevention of lethal septic shock. *Plant Biotechnol. J.* 9:22–31
92. Yeboah A, Cohen RI, Rabolli C, Yarmush ML, Berthiaume F. 2016. Elastin-like polypeptides: a strategic fusion partner for biologics. *Biotechnol. Bioeng.* 113:1617–27
93. Liu W, MacKay JA, Dreher MR, Chen M, McDaniel JR, et al. 2010. Injectable intratumoral depot of thermally responsive polypeptide-radionuclide conjugates delays tumor progression in a mouse model. *J. Control. Release* 144:2–9
94. Liu W, McDaniel J, Li X, Asai D, Garcia Quiroz F, et al. 2012. Brachytherapy using injectable seeds that are self-assembled from genetically encoded polypeptides in situ. *Cancer Res.* 72:5956–65
95. Asai D, Xu D, Liu W, Garcia Quiroz F, Callahan DJ, et al. 2012. Protein polymer hydrogels by in situ, rapid and reversible self-gelation. *Biomaterials* 33:5451–58
96. McDaniel JR, Weitzhandler I, Prevost S, Vargo KB, Appavou M-S, et al. 2014. Noncanonical self-assembly of highly asymmetric genetically encoded polypeptide amphiphiles into cylindrical micelles. *Nano Lett.* 14:6590–98
97. Schaal JL, Li X, Mastria E, Bhattacharyya J, Zalutsky MR, et al. 2016. Injectable polypeptide micelles that form radiation crosslinked hydrogels in situ for intratumoral radiotherapy. *J. Control. Release* 228:58–66
98. Williams RF, Gleason RE, Soeldner JS. 1968. The half-life of endogenous serum immunoreactive insulin in man. *Metabolism* 17:1025–29
99. Hui H FL, Merkel P, Perfetti R. 2002. The short half-life of glucagon-like peptide-1 in plasma does not reflect its long-lasting beneficial effects. *Eur. J. Endocrinol.* 146:863–69
100. Varanko AK, Chilkoti A. 2019. Molecular and materials engineering for delivery of peptide drugs to treat type 2 diabetes. *Adv. Healthcare Mater.* 8:1801509
101. Amiram M, Luginbuhl KM, Li X, Feinglos MN, Chilkoti A. 2013. A depot-forming glucagon-like peptide-1 fusion protein reduces blood glucose for five days with a single injection. *J. Control. Release* 172:144–51

102. Luginbuhl KM, Schaal JL, Umstead B, Mastria EM, Li X, et al. 2017. One-week glucose control via zero-order release kinetics from an injectable depot of glucagon-like peptide-1 fused to a thermosensitive biopolymer. *Nat. Biomed. Eng.* 1:0078
103. Amiram M, Luginbuhl KM, Li X, Feinglos MN, Chilkoti A. 2013. Injectable protease-operated depots of glucagon-like peptide-1 provide extended and tunable glucose control. *PNAS* 110:2792–97
104. Wang W, Jashnani A, Aluri SR, Gustafson JA, Hsueh P-Y, et al. 2015. A thermo-responsive protein treatment for dry eyes. *J. Control. Release* 199:156–67
105. Rizvi SAA, Saleh AM. 2018. Applications of nanoparticle systems in drug delivery technology. *Saudi Pharm. J.* 26:64–70
106. Lee TAT, Cooper A, Apkarian RP, Conticello VP. 2000. Thermo-reversible self-assembly of nanoparticles derived from elastin-mimetic polypeptides. *Adv. Mater.* 12:1105–10
107. Wright ER, Conticello VP. 2002. Self-assembly of block copolymers derived from elastin-mimetic polypeptide sequences. *Adv. Drug Deliv. Rev.* 54:1057–73
108. Wright ER, McMillan RA, Cooper A, Apkarian RP, Conticello VP. 2002. Thermoplastic elastomer hydrogels via self-assembly of an elastin-mimetic triblock polypeptide. *Adv. Funct. Mater.* 12:149–54
109. Dreher MR, Simnick AJ, Fischer K, Smith RJ, Patel A, et al. 2008. Temperature triggered self-assembly of polypeptides into multivalent spherical micelles. *J. Am. Chem. Soc.* 130:687–94
110. Simnick AJ, Valencia CA, Liu R, Chilkoti A. 2010. Morphing low-affinity ligands into high-avidity nanoparticles by thermally triggered self-assembly of a genetically encoded polymer. *ACS Nano* 4:2217–27
111. Shah M, Edman MC, Janga SR, Shi P, Dhandhukia J, et al. 2013. A rapamycin-binding protein polymer nanoparticle shows potent therapeutic activity in suppressing autoimmune dacryoadenitis in a mouse model of Sjögren's syndrome. *J. Control. Release* 171:269–79
112. Shi P, Aluri S, Lin Y-A, Shah M, Edman M, et al. 2013. Elastin-based protein polymer nanoparticles carrying drug at both corona and core suppress tumor growth in vivo. *J. Control. Release* 171:330–38
113. Sun G, Hsueh P-Y, Janib SM, Hamm-Alvarez S, MacKay JA. 2011. Design and cellular internalization of genetically engineered polypeptide nanoparticles displaying adenovirus knob domain. *J. Control. Release* 155:218–26
114. Hsueh P-Y, Edman MC, Sun G, Shi P, Xu S, et al. 2015. Tear-mediated delivery of nanoparticles through transcytosis of the lacrimal gland. *J. Control. Release* 208:2–13
115. Maeda Y, Mochiduki H, Ikeda I. 2004. Hydration changes during thermosensitive association of a block copolymer consisting of LCST and UCST blocks. *Macromol. Rapid Commun.* 25:1330–34
116. Arotçaréna M, Heise B, Ishaya S, Laschewsky A. 2002. Switching the inside and the outside of aggregates of water-soluble block copolymers with double thermoresponsivity. *J. Am. Chem. Soc.* 124:3787–93
117. Shih Y-J, Chang Y, Deratani A, Quemener D. 2012. "Schizophrenic" hemocompatible copolymers via switchable thermoresponsive transition of nonionic/zwitterionic block self-assembly in human blood. *Biomacromolecules* 13:2849–58
118. Yuan W, Zou H, Guo W, Wang A, Ren J. 2012. Supramolecular amphiphilic star-branched copolymer: from LCST–UCST transition to temperature–fluorescence responses. *J. Mater. Chem.* 22:24783–91
119. Weitzhandler I, Dzuricky M, Hoffmann I, Garcia Quiroz F, Gradzielski M, Chilkoti A. 2017. Micellar self-assembly of recombinant resilin-/elastin-like block copolypeptides. *Biomacromolecules* 18:2419–26
120. Dzuricky M, Xiong S, Weber P, Chilkoti A. 2019. Avidity and cell uptake of integrin-targeting polypeptide micelles is strongly shape-dependent. *Nano Lett.* 9: 6124–32
121. McDaniel JR, Bhattacharyya J, Vargo KB, Hassouneh W, Hammer DA, Chilkoti A. 2013. Self-assembly of thermally responsive nanoparticles of a genetically encoded peptide polymer by drug conjugation. *Angew. Chem. Int. Ed.* 52:1683–87
122. MacKay JA, Chen M, McDaniel JR, Liu W, Simnick AJ, Chilkoti A. 2009. Self-assembling chimeric polypeptide–doxorubicin conjugate nanoparticles that abolish tumours after a single injection. *Nat. Mater.* 8:993–99
123. Mastria EM, Cai LY, Kan MJ, Li X, Schaal JL, et al. 2018. Nanoparticle formulation improves doxorubicin efficacy by enhancing host antitumor immunity. *J. Control. Release* 269:364–73

124. Bhattacharyya J, Bellucci JJ, Weitzhandler I, McDaniel JR, Spasojevic I, et al. 2015. A paclitaxel-loaded recombinant polypeptide nanoparticle outperforms Abraxane in multiple murine cancer models. *Nat. Commun.* 6:7939
125. Bhattacharyya J, Ren X-R, Mook RA, Wang J, Spasojevic I, et al. 2017. Niclosamide-conjugated polypeptide nanoparticles inhibit Wnt signaling and colon cancer growth. *Nanoscale* 9:12709–17
126. Bhattacharyya J, Weitzhandler I, Ho SB, McDaniel JR, Li X, et al. 2017. Encapsulating a hydrophilic chemotherapeutic into rod-like nanoparticles of a genetically encoded asymmetric triblock polypeptide improves its efficacy. *Adv. Funct. Mater.* 27:1605421
127. Costa SA, Mozhdehi D, Dzuricky MJ, Isaacs FJ, Brustad EM, Chilkoti A. 2019. Active targeting of cancer cells by nanobody decorated polypeptide micelle with bio-orthogonally conjugated drug. *Nano Lett.* 19:247–54
128. Huang W, Rollett A, Kaplan DL. 2015. Silk-elastin-like protein biomaterials for the controlled delivery of therapeutics. *Expert Opin. Drug Deliv.* 12:779–91
129. Megeed Z, Cappello J, Ghandehari H. 2002. Genetically engineered silk-elastinlike protein polymers for controlled drug delivery. *Adv. Drug Deliv. Rev.* 54:1075–91
130. Xia X-X, Wang M, Lin Y, Xu Q, Kaplan DL. 2014. Hydrophobic drug-triggered self-assembly of nanoparticles from silk-elastin-like protein polymers for drug delivery. *Biomacromolecules* 15:908–14
131. Luginbuhl KM, Mozhdehi D, Dzuricky M, Yousefpour P, Huang FC, et al. 2017. Recombinant synthesis of hybrid lipid–peptide polymer fusions that self-assemble and encapsulate hydrophobic drugs. *Angew. Chem. Int. Ed.* 56:13979–84
132. Zheng L, Sundaram HS, Wei Z, Li C, Yuan Z. 2017. Applications of zwitterionic polymers. *React. Funct. Polym.* 118:51–61
133. Salmaso S, Caliceti P. 2013. Stealth properties to improve therapeutic efficacy of drug nanocarriers. *J. Drug Deliv.* 2013:374252
134. D'souza AA, Shegokar R. 2016. Polyethylene glycol (PEG): a versatile polymer for pharmaceutical applications. *Expert Opin. Drug Deliv.* 13:1257–75
135. Harris JM, Chess RB. 2003. Effect of pegylation on pharmaceuticals. *Nat. Rev. Drug Discov.* 2:214
136. Veronese FM, Pasut G. 2005. PEGylation, successful approach to drug delivery. *Drug Discov. Today* 10:1451–58
137. Banskota S, Yousefpour P, Kirmani N, Li X, Chilkoti A. 2019. Long circulating genetically encoded intrinsically disordered zwitterionic polypeptides for drug delivery. *Biomaterials* 192:475–85
138. Cao Z, Jiang S. 2012. Super-hydrophilic zwitterionic poly(carboxybetaine) and amphiphilic non-ionic poly(ethylene glycol) for stealth nanoparticles. *Nano Today* 7:404–13
139. Ladd J, Zhang Z, Chen S, Hower JC, Jiang S. 2008. Zwitterionic polymers exhibiting high resistance to nonspecific protein adsorption from human serum and plasma. *Biomacromolecules* 9:1357–61
140. Yin J, Li G, Ren X, Herrler G. 2007. Select what you need: a comparative evaluation of the advantages and limitations of frequently used expression systems for foreign genes. *J. Biotechnol.* 127(3):335–47