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Review

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Nanotechnological Approaches to Therapeutic Delivery Using Elastin-Like Recombinamers

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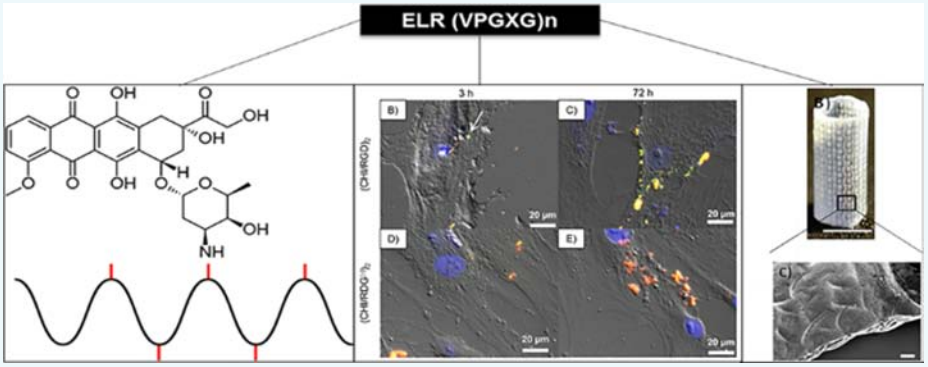

ABSTRACT: This Review discusses the use of elastin-like polymers and their recombinant version, elastin-like recombinamers, in drug-delivery systems. These macromolecules exhibit a number of interesting properties that are rarely found together in any other family of materials, especially extremely high biocompatibility, high bioactivity and functionality, complex yet fully controlled composition, and stimuli responsiveness. Appropriate design of these molecules opens up a broad range of different possibilities for their use in new therapeutic platforms. The first of these described herein is the use of ELRs in single-molecule devices as therapeutic entities in their own right. Subsequently, we describe how the self-assembly properties of these materials can be exploited to create nanocarriers and, eventually, microcarriers that are able to temporally and spatially control and direct the release of their drug load. Intracellular drug-delivery devices and nanocarriers for treating cancer are among the uses described in that section. Finally, the use of ELRs as base materials for implantable drug depots, in the form of hydrogels, is discussed.

1. INTRODUCTION

Besides the obvious essential role of pharmacology in the development of therapeutic agents, materials science also plays a pivotal role in this task. Thus, pharmacology aims to find new therapeutic molecules, whereas the field of biomaterials provides a platform for the development of a wide range of formats as delivery vehicles which, compared to the administration of free drugs, can provide advantages such as improved stability, solubility, and in vivo pharmacokinetics.[1,2]

Protein-based biopolymers are excellent candidates for the design of vehicles for drug-delivery purposes since their properties are determined exclusively by the physicochemical properties of their component monomers and their sequence.[3] The 20 chemically distinct natural amino acids can be combined in an almost infinite manner, thus providing high versatility and allowing a rational and precise design of protein-based materials to fulfill specific therapeutic requirements.

This Review is focused on describing the engineering of elastin-like polypeptides (ELPs) for therapeutic applications.[4−6] As their name implies, ELPs are polypeptides whose sequence is bioinspired by that found in natural elastin. Thus, the most widely used motif has the sequence $(VPGXG)_n$, where the guest residue X is any amino acid except proline and n symbolizes the number of pentapeptide repeats in the ELP, although additional motifs such as VPG, VPGG, GVGVP, IPGVG, or VAPGVG have also been explored.[7] In order to ensure strict control over the sequence, chain complexity, and monodispersity, recombinant DNA technology has been implemented to bioproduce this class of materials.[8] Indeed, a new term, namely, elastin-like recombinamers (ELRs), has been coined to refer to those ELPs produced using genetic engineering techniques.[7,9,10]

Besides their specific properties with regard to a particular design, the mimicry between elastin and ELRs can also be seen in their similarity with regard to other properties, such as their biocompatibility, mechanical properties, and stimuli-responsive

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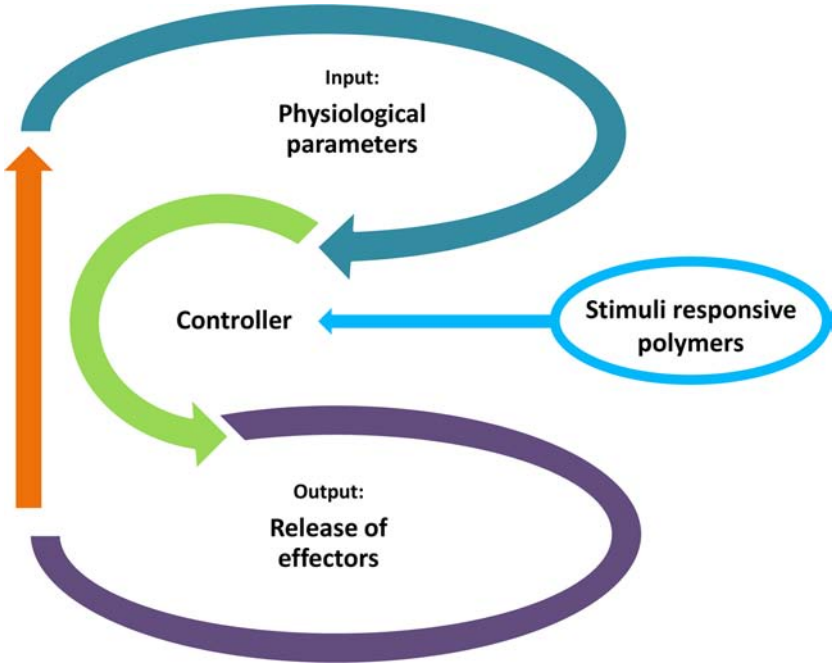

Figure 1. Scheme showing the physiological homeostatic feedback exerted by the human body. Such a loop arrangement ensures strict control over the physiological parameters. Recent trends in drug delivery try to emulate this loop arrangement by exploiting the environmental sensitivity exhibited by stimuli-responsive polymers. Note that the stimuli-responsive polymers alone are not able to complete the negative feedback loop as they need to be combined with drugs (effectors) to do so.

behavior. This behavior is the result of a phase transition from soluble to insoluble that the ELR experiences in response to an environmental stimulus such as pH, salt, or temperature, among others.[11] The most common variable exploited to achieve such a transition is temperature, which has led to definition of the parameter "Tt" as the specific temperature above which the polymer chain remains hydrated, and below which the ELR chain folds hydrophobically and assembles to form a separate phase. In this folded state, the polymer chains adopt a dynamic, regular, nonrandom structure known as a β-spiral.[12,13] Indeed, the fact that the polymer transforms thermal energy into mechanical work has led these polymers to be described as molecular machines[11] and the overall process as an inverse temperature transition (ITT).[14,15]

Such stimuli-responsive behavior is especially relevant when engineering smart devices that are able to sense their environment and response to changes in it,[16] since this closely mimics the behavior of the human body, which is able to detect surrounding stimuli, also known as "inputs", and elaborate a response, or "output", in order to exert close control over the resulting physicochemical process and correct potential imbalances. It is important to note that close control over the physicochemical process by means of a feedback loop requires the ELR to be combined with effectors or drugs (Figure 1).

All the features listed above have provided a significant impetus to the development of various elastin-based platforms as carrier systems for the delivery of a variety of payloads (e.g., drugs, proteins, peptides, nucleic acids) in a variety of formats (hydrogels or depots, nanoparticles, monomers) over the past few years. As such, this Review focuses on describing the most significant approaches in this field.

2. MONOMERIC ELRS

Monomeric elastin-like recombinamers can be described as single recombinant elastin-based polypeptides adapted for use in therapeutics. They can be fused to therapeutic proteins in a recombinant manner for use as purification tags, as soluble delivery systems or as pharmacokinetic enhancers themselves, either by chemical conjugation or by fusion-protein recombinant expression of the drug.

2.1. ELRs as Purification Tags. One of the major issues in the downstream processes for obtaining recombinant proteins for therapeutic purposes is their high cost due to the need for chromatographic and other costly and time-consuming product-purification techniques.[17] To address this problem, ELRs can be used as purification tags by taking advantage of a simple, cheap, and high-yield purification approach that relies on the ITT described above, in which the recombinamer can be obtained pure after several heating and cooling cycles following by centrifugation in a process called inverse transition cycling (ITC). In addition, the ELR-based polypeptide can protect the protein/peptide from proteolytic degradation and from unfolding during the purification process. Further separation requires excision of the ELR tag and isolation of the target therapeutic protein, which is usually achieved by an additional heating and centrifugation step in which the ELR precipitates and the drug remains soluble in the supernatant.[18−20] This method is only useful when the target protein is thermally stable at mild temperatures (37−42 °C), if a hydrophobic ELR with a low ITT is fused to it, or if salts are used in the ITC steps in order to lower the ITT.

Under this premise, several approaches, which mainly differ with regard to the tag cleavage strategy, have been developed to enhance the downstream process of recombinant drugs (Table 1). One of these methods involves the inclusion of a self-processing module (SPM) from *Neisseria meningitides* FrpC protein, which undergoes autocatalytic cleavage between the Asp^{414} and Pro^{415} peptide bond at physiological calcium ion concentrations.[21] This SPM has been successfully inserted into ELR constructs fused to green fluorescent protein (GFP), to

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Table 1. Production Yields for Different Proteins after Excision from the ELR Tag Using Diverse Approaches

excision approach	target protein	yield of the final product[a] (mg/L)	references
FrpC	GFP	1.1 to 36	Liu et al.[22]
	pFc		
	HBD3[b]		
SrtA	TRX	35	Bellucci et al.[36]
	GFP	28	
	TNFα	16	
	TRAIL	9	
Intein	β-Gal	122.3	Banki et al.[25]
	Catalase	79.8	
	GFP	110.2	
	MBP	46.4	
	OPH-S5	83	Fong et al.[26]
	CM4[b]	0.6	Shen et al.[32]
	HβD4[b]	1.8	
Hydroxylamine cleavage	Hal18[b]	1.7	Hu et al.[29]
Enterokinase proteolysis	CAD[b]	12	Yang et al.[35]

[a]mg of final product per L of culture medium. [b]Antimicrobial peptides (AMPs).

the Fc portion of porcine IgG (pFc), and to human β defensin 3 (HBD3). After expression in *Escherichia coli*, the resulting ELR fusion polypeptides were purified by ITC and the product obtained by cleavage in a Ca^{2+} solution. Production yields ranged from 1.1 to 36 mg/L and purities from 90% to 100% of the final cleaved products.[22]

A similar methodology involves the addition of a self-cleaving intein sequence involved in protein splicing.[23] This sequence self-cleaves when dissolved in a saline pH 6.0 buffer at room temperature and was first evaluated for use in bioseparations by Wood et al.[24] It was subsequently inserted into an ELR by this same group and the ELR-intein gene was fused to those coding for different proteins, such as β-galactosidase, catalase, GFP or maltose binding protein (MBP), among others, to finally obtain highly pure products with specific activity when expressed in *E. coli* after ITC and cleavage reaction.[25] This same strategy was used to purify ELR-intein-tagged proteins in high cell density *E. coli* fed-batch fermentations[26] and for the production of recombinant *E. coli* RNA polymerase.[27]

Various antimicrobial peptides (AMPs) have been specifically expressed fused to an ELR tag for easier purification. Thus, halocidin18 (Hal18), which possesses strong antimicrobial activity against *E. coli* and *Micrococcus luteus*, was cloned into an ELR amino acid sequence and expressed in *E. coli*, with 69 mg/L of the fusion product being obtained. Subsequent excision of Hal18 was achieved by the addition of hydroxylamine cleavage (between asparagine and glycine residues)[28] reaction buffer and incubation at 55 °C for 24 h, finally giving a recovery of approximately 47% of the product (1.7 mg/L) and a moderate purity of 60%.[29] Alternative AMPs, namely, moricin CM4 and human β-defensin 4 (HβD4), were fused to an ELR tag, expressed in *E. coli* and cleaved due to the inclusion of an intein self-cleaving sequence, although the final yield of the target protein was not high (0.6 mg/L for CM4, 5.5 mg/L in the case of the lone recombinant protein,[30] and 1.8 mg/L for HβD4, compared to 5 mg/L of the recombinant AMP[31]).[32] Furthermore, the antimicrobial peptide cecropin AD (CAD) was fused to a cationic ELR (selected from previous studies[33]) for ITC purification, and a high purity CAD was obtained after expression in *E. coli* with a 12 mg/L yield (compared to 11.2 mg/L for the recombinant CAD[34]). In this case, separation of the ELR from the target AMP was performed by proteolysis with enterokinase.[35]

Another approach for the cleavage of recombinant therapeutic proteins, which involves the inclusion of a transpeptidase recognition sequence (LPETG and LPGAG) in the ELR fusion polypeptide, has been described recently. This domain is recognized by Sortase A (SrtA), an enzyme found in *Staphylococcus aureus*, which cleaves the target protein from the ELR, and can be also expressed fused to an ELR to improve its production and purification. Furthermore, both enzyme and target protein can be expressed in the same molecule. These methods have allowed different recombinant proteins, including thioredoxin (TRX), green fluorescent protein (GFP), and the pharmaceutically relevant proteins soluble murin tumor necrosis factor α (TNFα) and soluble human tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), to be obtained in yields of 35, 28, 16, and 9 mg/L of culture medium, respectively.[36]

2.2. Therapeutic Monomeric ELRs. Therapeutic elastin-like recombinamers are a promising tool for the design of recombinant drugs as this recombinant system adds new features to the drug itself in terms of specific delivery and improved pharmacokinetics. There are two different approaches for this purpose: chemical conjugation of the drug to the ELR via reactive groups, which allows the use of protein and nonprotein drugs, or engineering of fusion recombinamers (ELR-drugs) in the case of protein-based therapeutics (Figure 2).

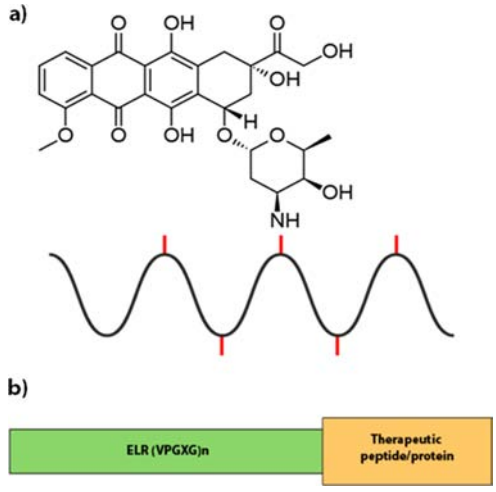

Figure 2. Schematic representation of (a) ELR-Dox bioconjugated via lysine or cysteine amino acids (represented as a red line) and (b) a therapeutic fusion recombinamer.

2.2.1. Chemical Bioconjugates. Different ELRs suitable for covalent bioconjugation to other therapeutic molecules have been designed. In every case, chemical conjugation is achieved by modifying reactive groups in the side chains of various amino acids, such as the thiol group in cysteine or the ε-amino group in lysine, thereby introducing cross-linking sites for different therapeutic molecules. The fact that a single ELR construct can be used in this case results in a less time-consuming strategy, as no molecular biology techniques have to be applied and the purification process does not need to be

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optimized. Furthermore, nonprotein drugs can also be used in this approach.

One of the most widely used molecules for chemical conjugation to ELRs is the chemotherapeutic agent doxorubicin (Dox). In a first attempt, this drug was conjugated by modification of an amine group from a lysine amino acid residue in the ELR to obtain a free maleimide group. A doxorubicin−hydrazone complex, which is cleavable because of the acid-labile nature of this hydrazine bond, was subsequently added to react with the maleimide group and achieve bioconjugation. In vitro studies regarding the cytotoxicity and subcellular localization of the conjugated drug showed a similar level of squamous cell carcinoma (FaDu) cell death (less than 10% survival after 72 h of treatment) when comparing the free and the conjugated drugs, but a different localization, with the drug itself mostly being internalized in the nucleus and the ELR-doxorubicin being dispersed throughout the cytoplasm, thus suggesting a different cell death pathway in each case.[37] In another experiment, doxorubicin was conjugated via cysteine residues and intracellular cleavage of the drug was achieved by either an acid-labile hydrazine bond[38] or an enzymatic reaction.[39] In this case, a Tat domain, a cationic cell-penetrating peptide (CPP) derived from the trans-activating protein found in HIV-1, was fused to the ELR to enhance cellular uptake, along with a GFLG tetrapeptide linker to act as a substrate for lysosomal cathepsin proteases, thus allowing release of the drug after endocytosis. The cytotoxicity of this "smart" system was tested in vitro, and the results showed that ELR-Dox was cytotoxic toward both non- and Dox-resistant carcinoma cell lines, being able to bypass the P-glycoprotein drug efflux that confers drug resistance. The relative resistance (obtained by comparing the IC_{50} values of non- and resistant cell lines) with respect to free Dox was 67.6% in the case of drug-resistant uterine sarcoma cells and 31.9% for breast cancer cells, while these values were 0.9% and 1.4%, respectively, for ELR-Dox.

An alternative approach for the delivery of drugs into target tissues, mainly solid tumors, is to bioconjugate them to ELRs that form aggregates above their transition temperature, this temperature lying between normal body temperature and that in a locally heated region (40−44 °C). This approach, which is known as hyperthermia, could help to localize the drug inside the tumor. Two different ELRs, one with a transition temperature of about 41 °C and the other with a transition temperature of more than 55 °C, have been designed and synthesized with this premise to compare the effect of temperature responsiveness and aggregation, or lack of it, on the tumor. These ELRs were fluorescently labeled and observed by in vivo fluorescence video microscopy after inoculation into a tumor region in mice. It was seen that the thermoresponsive ELR showed a 2-fold increase in accumulation in heated tumors compared to the nonresponsive ELR, thereby suggesting its potential use in hyperthermia-based drug delivery when conjugated to chemotherapeutic drugs.[40] Similar results were found in another study in which a 2-fold higher uptake of the labeled hyperthermally aggregated ELR by different carcinoma cell lines when compared to the control soluble one was observed, although some differences in uptake were seen between cell lines. The preferential internalization of particles with a size of around 100 nm was also observed by confocal microscopy and flow cytometry.[41]

As in the case of soluble conjugated ELRs, and taking advantage of the thermal responsiveness of some of these polypeptides, doxorubicin has been chemically bioconjugated to different ELRs in order to achieve local accumulation of this chemotherapeutic agent. Thus, the same system and chemical reaction as that described above, namely, Tat-ELP-GFLG-Dox,[39] was used in hyperthermia treatment and found to provide a 20-fold enhancement in the cytotoxicity toward MES-SA uterine sarcoma cells, compared to nonthermal inducement, with a cytoplasmic distribution of the drug. Addition of the Tat sequence permits this higher uptake and efficiency of the drug compared with the values found in previous studies with nonconjugated labeled ELRs (see above).[42] A hydrazone derivative of doxorubicin was also bioconjugated to an ELR including another CPP (SynB1-ELR) via C-terminal cysteines and subsequently released in lysosomes after internalization because of its acid-sensitivity. In vitro results in E0771 breast cancer cells showed a cytoplasmic distribution of the labeled drug, while in vivo experiments carried out in tumor-implanted mice treated with hyperthermia resulted in an increase in the drug half-life, no detectable levels of doxorubicin in the heart (which is one of the most dangerous side effects of the free drug) and complete tumor growth inhibition; this effect was only moderate in the case of the free drug.[43] A different chemotherapeutic drug used in cancer treatment, known as paclitaxel, which arrests the cell cycle and induces apoptosis, was similarly conjugated to the cell-penetrating peptide fusion ELR SynB1-ELR by coupling hydrazine-derivatized paclitaxel and a thiol-reactive maleimide group from a C-terminal cysteine residue, resulting in improved drug solubility below the transition temperature of the ELR. This construct was further tested in vitro by culture with the MCF-7 breast cancer cell line, inducing ELR aggregation by hyperthermia and achieving similar results to those obtained with conventional paclitaxel. However, even paclitaxel-resistant MCF-7 cells were killed with this treatment, thus suggesting a way to overcome drug resistance.[44]

2.2.2. Fusion Recombinamers. Alternatively, and taking advantage of the enormous potential of recombinant DNA techniques, therapeutic proteins can be fused to an ELR backbone in order to improve the expression, purification, and, additionally, delivery of pharmacokinetics thereof.

Many anti-inflammatory proteins, such as antibodies that recognize pro-inflammatory cytokines, have been produced recombinantly fused to ELRs, a method called ELPylation (for elastin-like polypeptide). This approach allows us to take advantage of both the ITC purification system and the improved stability of the therapeutic protein once in the bloodstream. Proof-of-concept studies in this regard were carried out with different proteins fused to an ELR in plants, with satisfactory results being obtained in terms of expression and purification.[45,46] An anti-human TNF was subsequently designed and integrated into an expression system fused to an ELR using this technique. Production was performed in *Nicotiana tabacum* plants and the chimera, which was found to have similar biological bioactivity to the single antibody when tested in both in vitro cell cultures and in vivo, was purified by several heating and cooling steps. However, a marked improvement was found in terms of serum half-life, which increased from 28 min to 11 h (24-fold).[47] In a similar manner, IL-10[48] and a HIV-neutralizing antibody[49] were engineered into an ELR-carrying vector for expression in tobacco plants and found to have a similar bioactivity to the native proteins. In a similar manner to the way in which whole proteins have been used in the design of fusion therapeutic drugs, peptides have been fused to ELRs to provide the benefits

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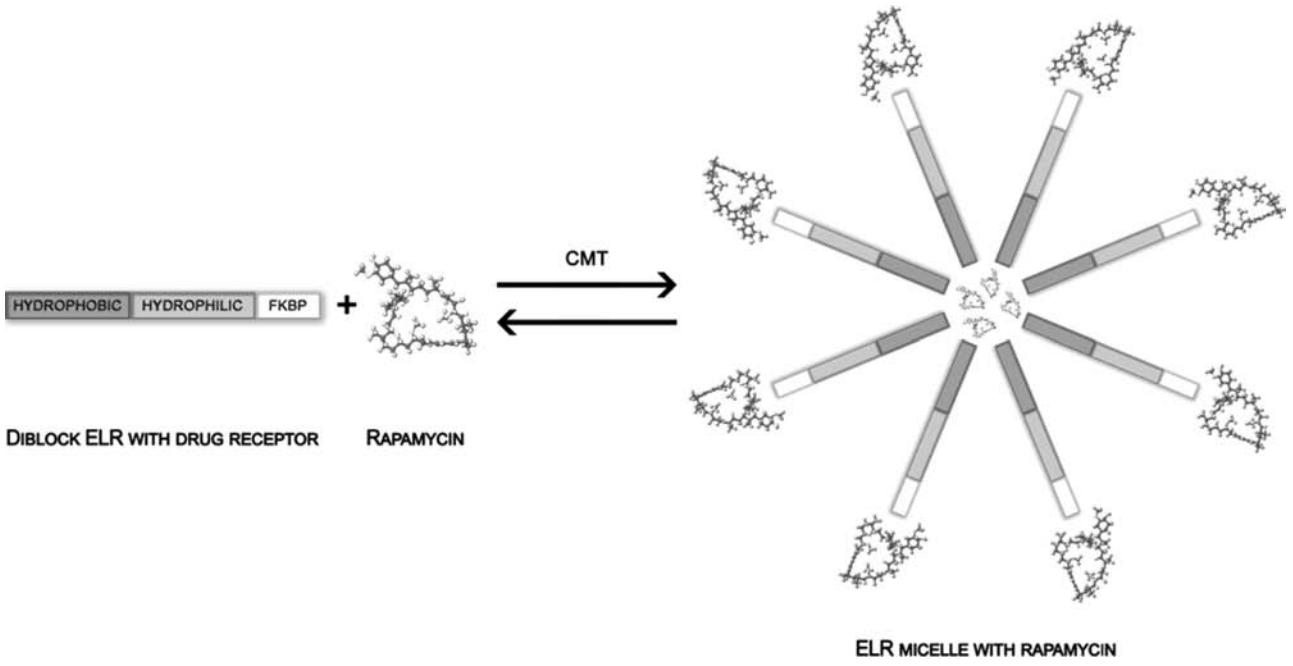

Figure 3. ELR diblock functionalized with FKBP. FKBP was bound at the C-t by genetic engineering techniques. The diblock binds to rapamycin via the binding protein FKBP with high affinity. Above the CMT the diblock adopts its micelle conformation, increasing the loading capacity for rapamycin and exposing it on the surface.

explained above. In one case, AP1, a ligand for the IL-4 receptor that is highly expressed in tumor cells and which amplifies the expression of some antiapoptotic proteins, thereby preventing drug-induced cancer cell death, was fused to a $(VPGVG)_n$ ELR containing six repetitions of the AP1 peptide amino acid sequence. This chimera was found to bind to the IL-4 receptor in vitro and in vivo by way of fluorescent labeling of the AP1-ELR, and also exhibited tumor accumulation.[50]

Another recent application, in this case for the treatment of dry eyes, was developed by fusing a VPGVG-based ELR to lacritin (Lacrt), a protein component of human tears with pro-secretory activity in the lacrimal gland. This construct allows precipitation of the protein and was found to be a good approach to overcoming the limitation of rapid tear turnover when administrating single Lacrt when tested in vivo, thus becoming a potential delivery system for the treatment of dry eyes.[51]

As in the case of chemically bioconjugated drug-ELR constructs, fusion recombinamers have also been designed in order to achieve tumor accumulation by hyperthermia by joining ELRs to different tumor-targeting drugs. One of the first attempts in this field involved fusion of a region of c-Myc (H1) known to inhibit c-Myc transcriptional function, together with the cell-penetrating peptide penetratin, to a thermally responsive ELR to enhance cellular uptake. The results showed a 2-fold increase in the antiproliferative properties of the thermoresponsive recombinamer in the MCF-7 breast cancer cell line when compared to a control nonaggregated recombinamer after hyperthermia.[52] This same structure was fused to different CPPs to perform a compartment-directed uptake, with maximum cell death being achieved when using Bac, a sequence that directs aggregates to the cell nucleus.[53] Subsequent studies with a very similar construct were performed using this recombinamer as a therapeutic agent for the treatment of glioblastoma in vivo in rats. The results showed an 80% reduction in tumor volume after hyperthermia-induced aggregation; therefore, this approach was suggested to be an alternative for drug delivery to brain tumors.[54] However, experiments regarding the cellular uptake efficiency and retention of ELRs in solid tumors after hyperthermia were only performed recently. These studies showed that larger ELRs are better when these properties are taken into account.[55]

Another peptide, in this case derived from lactoferrin (L12), has been fused to a thermally responsive ELR and the Tat domain and tested for the treatment of pancreatic cancer in vitro. The results showed a high cytotoxicity of the recombinamer when cultured with MIA PaCa-2 pancreatic adenocarcinoma cells after thermally induced aggregation in comparison with a control nonaggregated fusion ELR.[56] Other studies have used the peptide p21, which arrests the cell cycle, to prevent cell proliferation and therefore as a potential drug for the treatment of cancer. One of the first ELR-p21 recombinamers, which was combined with Bac CPP, showed internalization of ELR aggregates and further antiproliferative effects by inhibition of the cell cycle in SKOV-3 ovarian cancer and MCF-7 breast cancer cells.[57] An analogous fusion recombinamer was combined with gemcitabine, a chemotherapeutic agent used in the treatment of pancreatic cancer, to enhance the antitumor effect while lowering the drug dose to reduce its side effects. The results showed cytotoxicity in MIA PaCa-2 and Panc-1 pancreatic cancer cell lines and tumor growth inhibition in mice in vivo after hyperthermia.[58]

3. DRUG DELIVERY USING ELR-BASED PARTICLES

3.1. Self-Assembled ELRs for Nano- and Microparticle Synthesis. The recombinant origin of ELRs allows absolute control over their physical and chemical features, such as functionality, biocompatibility, and self-assembly, among others.[59−63] This self-assembly behavior can be induced by the design of the ELR construct itself, conjugation of a drug, addition of charged molecules, or mediation by an external stimulus, such as temperature, pH, and so forth.[64−70]

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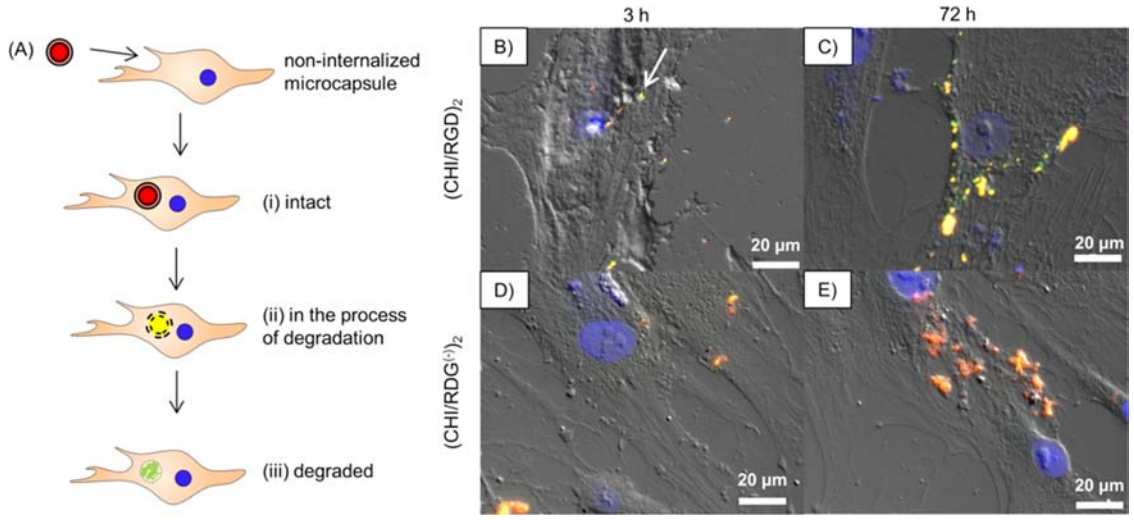

Figure 4. (A) Degradation of DQ-ovalbumin inside the cell. Fluorescence and DIC microscopy images of $(CHI/ELR\text{-}RGD)_2$ and $(CHI/ELR\text{-}RDG(-))_2$ microcapsules loaded with DQ-ovalbumin incubated with hMSCs for (B and D) 3 h or (C and E) 72 h and DAPI staining. The arrow in B indicates a bright green spot. A shift from red/orange to green/yellow indicates a transition from a fully intact to a degraded cargo.

The thermosensitivity of ELRs makes them ideal for constructs made from different hydrophobic and hydrophilic blocks, constituting what are known as "block copolymers". For example, in an aqueous solution of a diblock ELR, a dehydration process and decrease in the polarity of this block triggers a conformational change of the whole ELR diblock from unimer to micelle above the transition temperature of the hydrophobic part. Hence, the hydrophobic block is located inside the micelle, thereby providing stability to the complex, whereas the hydrophilic block is positioned outward in contact with surrounding water. When the temperature is increased above the Tt of the hydrophilic block, it dehydrates and the micelles aggregate. The temperature at which the transition from a soluble ELR diblock to a micelle occurs is called the critical micelle temperature (CMT). This CMT can be controlled by varying the length and composition of the hydrophobic block, whereas the size of the micelle can be controlled by varying the ELR length and the hydrophilic-to-hydrophobic block ratio.[71] The high versatility in the design of ELR diblocks even allows control over whether self-assembly results in micelles or hollow vesicles by varying the block arrangement and length. This conclusion was reached in studies with different ELR constructs comprising glutamic acid and alanine blocks, as described by Martin et al.[60] ELR diblocks can be functionalized for different purposes by genetically inserting protein domains into their sequence. Thus, a binding protein such as FKBP (*Plasmodium falciparum* FK506-binding protein), which targets the drug rapamycin, was inserted into the hydrophilic domain of the ELR diblock and used to enhance the affinity of the ELR for this drug (Figure 3). This system was tested in vitro in a breast cancer model cell line and was found to increase the loading capacity and release, and therefore the anticancer activity, of the drug.[72] Additionally, ELR diblocks were designed with the knob protein domain from adenovirus to improve the cellular internalization of the complex. The knob domain binds to the overexpressed adenovirus receptor (CAR), which is widely located in hepatocytes and acinar cells. Formation of the ELR micelle above a certain Tt, with a size of about 40 nm, caused the knob domains to be exposed outward. The addition of protein domains to the ELR diblock facilitated its interaction and internalization inside the hepatocytes compared with the nonmodified ELR.[73]

ELRs can also be self-assembled chemically by cross-linking methods, forming thermoresponsive microgel capsules (MCs) that can be used for drug-delivery purposes. Chemical cross-linking maintains the shape of the microspheres, while the pore size can be reversibly changed by the application of external stimuli, thus controlling drug delivery. Na et al., for example, used a mixture of ELR and albumin at different mass ratios and cross-linked the ELR to both enhance shape stability and control the structure of the surface when changing the temperature.[74] Above the Tt of the ELRs tested, the release of albumin and prednisone acetate as model drugs increased due to the formation of a porous structure, whereas they were released slowly below this Tt. Gradual release was found in the temperature range 20−40 °C. Subsequently, Cheng et al. created ELR/BSA MCs using a two-step cross-linking method[75] and evaluated the ability of these MCs to release a drug using two model molecules with similar molecular weights, namely, rhodamine B and the negatively charged FITC. The results showed a charge-independent release of both molecules from the microcapsules. Additionally, the high loading and release speed of the cargo molecules was confirmed upon increasing the temperature above the Tt of the ELR. In contrast, narrowing of the pores, and therefore no release of the cargo, was observed when decreasing the temperature below the Tt. Both studies show the potential of ELR microcapsules self-assembled using cross-linking methods and their ability to encapsulate and release drugs in response to temperature changes.

In addition, the biocompatible nature of ELRs means that they have been used in the formation of microparticles following layer-by-layer strategies for the encapsulation and delivery of a model cargo protein into cells. Thus, for example, a layer-by-layer approach was employed to create microparticles made from alginate-chitosan and chitosan-ELR as a proof-of-concept for the methodology.[76] In a more recent work, multilayered coated capsules made of chitosan and an ELR functionalized with bioactive sequences were used as building blocks and assembled on spherical particles of calcium carbonate.[77] The ELR was designed to contain the RGD

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sequence, thereby improving recognition of the particle by cell adhesion integrins. The resulting multilayered particles, with a size of about 3−4 μm, loaded with the model protein ovoalbumin were incubated with hMSCs for 72 h. After this time, noncytotoxic effects were observed. Additionally, degraded DQ-ovoalbumin, which was used to monitor the fluorescence changes in the cargo, showed internalization and higher degradation when encapsulated in RGD-functionalized ELR-chitosan microparticles and was found to be located mainly in the cytoplasm (Figure 4). This strategy paves the way to the creation of protein-based drug-delivery architectures made from ELRs, thereby conferring versatile design alternatives and highly biocompatible properties.

Self-assembly can also be triggered by the covalent attachment of hydrophobic drugs, such as doxorubicin or paclixatel.[65,66] Thus, Chilkoti's group conjugated a high-molecular-weight ELR to doxorubicin via the cysteine residues genetically incorporated at one end of the polypeptide. The hydrophobic drug triggered self-assembly of the recombinamer into nanoparticles with a diameter of 40 nm in which the drug was located at the core and surrounded by the hydrophilic ELR. In vivo assays with a colon cancer mouse model showed the induction of almost complete tumor regression, with a 4-fold higher maximum tolerated dose than for the free drug after a single administration and promoted by the ELR nanoparticles.[66]

The addition of charged molecules, such as nucleic acids, in a noncovalent manner can also induce the self-assembly process. Thus, negatively charged plasmid DNA can interact electrostatically with a specifically designed ELR for use in gene delivery. In one example, an ELR containing a cationic oligolysine tail (K8-ELP (1−60)) was complexed with pDNA, with the results showing a good complexation ability and stability of the polyplexes for the N/P ratios tested. In terms of transfection, ELR-pDNA polyplexes with a size of about 115 nm and encoding for GFP (green fluorescent protein) were able to transfect the MCF-7 cells tested, although some cytotoxic effects were observed,[78] probably due to the oligolysine tail. A recent study of ELRs designed with the functional motifs penetratin, LAEL fusogenic peptide, and imidazole groups showed the formation of stable polyplexes with a size of around 200 nm and a zeta potential of +24 mV. Additionally, hemocompatibility and cytotoxicity assays confirmed the innocuous nature of these ELRs. The ELR containing the LAEL peptide was found to be the best option for cellular uptake and transfection of the ELRs tested.[67] These results demonstrate the potential of ELR for gene therapy applications.

Finally, the aforementioned external temperature stimulus can trigger the self-assembly of ELRs by virtue of their smart nature: when the temperature increases, aggregation phenomena in ELRs lead to a spontaneous coacervation process. ELRs in the coacervation phase can be exploited and used as carriers for prolonged drug delivery. In this regard, studies with a keratinocyte growth factor (KGF)-ELR fusion polymer suggested the possible use of this polymer for the treatment of chronic wounds, with coacervation from the ELR component occurring at 37 °C even when the growth factor was bound to the ELR. If the coacervate could be located in the damaged tissue, this would allow the progressive administration of KGF, thereby enhancing granulation and re-epithelization when compared with a fibrin gel containing KGF in genetically diabetic male mice.[68] Alternatively, in vitro assays with C2C12 cells showed that another type of ELR containing $(VPAVG)_{220}$, which formed nanoparticles with a size of 237 nm, was able to encapsulate significant amounts of both BMP-2 and BMP-14 (bone morphogenetic proteins). In this case, the release process in vitro was associated with a two-phase profile characterized by a burst delivery during the first 24 h and followed by a sustained and slower release over 14 days.[69] This slow release of both KGF and BMP factors suggests the significant potential of ELR coacervates for both tissue regeneration applications and for the release of other types of molecules, such as therapeutic drugs.

Alternatively, self-assembled ELRs have been used as nanovaccines for tuberculosis.[79] The incorporation of an antigenic molecule from *M. tuberculosis* at the hydrophilic terminus of the ELR diblock $E_{50}I_{60}$ (comprising a hydrophilic block based on glutamic acid as guest residue (E_{50}) and a hydrophobic block based on isoleucine (I_{60})) using recombinant techniques resulted in the formation of Ag-$E_{50}I_{60}$. This construct was found to reversibly self-assemble into highly biocompatible, multivalent, monodisperse, and stable nanoparticles. These particles were able to trigger an innate immune response following by an adaptative Th2 response due to the presence of IL-5 and up-regulation of IgM and IgG in in vivo assays. The control used (diblock ELR or Ag alone) did not trigger any immunomodulatory response. These results support the use of this kind of ELR diblock construct as a potential antigen carrier for the development of more effective vaccines.

3.2. Self-Assembled ELR Particles and Hyperthermia.

The thermal sensitivity provided by ELR-based materials is a very attractive feature from the point of view of cancer therapy as the tumor can be heated externally and, consequently, the ELR can undergo a phase transition in the damaged tissue locally.

Tumor tissues are different from normal tissues, with components such as tumor vessels having been reported to possess larger intercellular pores than unaffected vessels. Additionally, a greater accumulation of lipids and macromolecules from the tumor cells occurs in cancer due to restricted lymphatic drainage. This phenomenon, known as the enhanced permeability and retention (EPR) effect, together with the local application of mild-hyperthermia (40−44 °C)[80] as adjuvant treatment for cancer therapy, can be taken advantage of by using thermosensitive materials such as ELRs. ELR diblocks comprising a hydrophobic block with a Tt > 42 °C that show self-assembly behavior from unimer to micelle when local mild-hyperthermia conditions are applied have been previously studied with regard to their physical properties and in vitro behavior. Additionally, the incorporation of different functional motifs has been shown to maintain the natural behavior of ELR diblocks and improve their action in mild hyperthermia. By way of illustration, when the RGD cell-adhesion motif was included in an ELR sequence comprising a hydrophilic block (containing valine, glycine, and alanine as guest residues in a 1:7:8 ratio) and a hydrophobic block (valine), the self-assembly temperature was maintained.[81] Additionally, an effect known as multivalency of the RGD was observed in the micelle conformation in response to the external application of hyperthermia, thereby constituting a "tunable thermal switch". This multivalency favors the uptake and binding of ELR diblocks, especially RGD-ELR-64/90, in the K562 leukemia cells tested.

Other ELR diblock constructs based on the same amino acid sequence mentioned above, but differing in the hydrophilic and

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hydrophobic block lengths, have been fused to two single domain proteins, namely, thioredoxin (Trx) and a fibronectin type III domain (Fn3) that binds the $\alpha_v\beta_3$ integrin. Binding of these domain proteins did not alter the micelle formation triggered by the ELR, and the resulting ELR diblocks functionalized with either Trx or Fn3 exhibited a size of 24−37 nm. One of these diblocks, named ELR-96−90, which comprises a hydrophilic block of 96 pentapeptides and a hydrophobic block of 90 pentapeptides fused to Fn3, showed bioactivity, enhancing targeting, and uptake by integrin-overexpressing K562/$\alpha_v\beta_3$ cells.[82] Additionally, the design of more complex self-assembled systems for drug delivery under mild hyperthermia conditions was also attempted. To this end, ELR diblocks functionalized with cell-penetrating peptides (CPP), as internalization motifs, and a drug payload were designed and named "nanopeptifiers".[83] The construct was genetically engineered from the BH3 peptide (derived from the proapoptotic Bak protein) located at the N-terminal end separated by a RVRR peptide acting as linker to the ELR diblock and followed by the CPP at the C-terminus (named Arg8-ELR_{BC}-cBH3). In this manner, the BH3 peptide load was sequestered in the micelle core upon self-assembly. The RVRR peptide was chosen due to its ability to be cleaved by furin and cathepsin B proteases, thus allowing intracellular release of the peptide drug following endocytic uptake. The diblock self-assembled from the unimer to the micelle under mild hyperthermia conditions (42 °C), with the CPPs being located on the outer surface of the micelle in high density. The tunable switching ability of CPP density in ELR diblock constructs had already been evaluated in earlier studies.[84] The cellular uptake confirmed the tunable nature of intracellular delivery by thermally triggered CPP-ELR diblock micelle assembly and release of the BH3 peptide. The bioactivity was assessed by activation of caspase-3 involved in the apoptosis processes. Thus, when a proapoptotic peptide was present in the nanopeptifier construct, the platform provided a cytotoxic switch that induced apoptosis only when the ELR diblock was self-assembled.

In summary, ELRs are stimulating materials of significant interest for biomedical applications, as their recombinant nature allows total control over the architecture and self-assembly features, thus allowing the introduction of sensitivity to external stimuli such as temperature. Novel applications in cancer therapy, including thermotherapy, are excellent examples of the use of this kind of naturally inspired biomaterials.

4. HYDROGELS AND DEPOTS FOR DRUG DELIVERY

Controlled-release systems usually require the use of a support or scaffold that acts as a drug reservoir, thus allowing sustained release of the therapeutic agent and avoiding "peak and valley" profiles inside the body.[85] Peptide-based hydrogels are gaining increasing attention for drug-delivery purposes thanks to the wide range of design possibilities conferred by the almost infinite number of possible combinations of amino acids. In other words, the structural features and functionalities of the gel network can be easily tailored by carefully engineering the peptide sequence.[86,87] Within this framework, elastin-based hydrogels are promising candidates for use in the construction of drug-delivery devices because of their protein-based nature and the inherent properties displayed by this class of materials, as highlighted in the Introduction. Thus, appropriately designed ELRs display a sol state below body temperature (37 °C), forming a viscous coacervate (depot) or hydrogel when implanted in the body. This ability to form a liquid-like state below Tt makes mixing with therapeutic agents efficient and extremely simple. Furthermore, implantation is painless and application is not restricted to accessible areas. Moreover, the injection can be applied directly at the target site, thus helping to extend local drug exposure while minimizing systemic side effects.[88] The protein nature of many pharmaceuticals paves the way for their inclusion into ELR-based devices both inside the ELR depot and bound to the ELR molecule.[89,90] In addition to their utility as drug reservoirs, ELR have further roles, such as macromolecular carriers, and it has even been speculated that they may act as a shield, protecting the therapeutic agent against protease attack.[91]

With regard to their ability to act as a macromolecular carrier, a system comprising a glucagon-like peptide-1 (GLP-1) fused to an ELR has been developed to achieve glucose control and therefore potential applications in the treatment of diabetes. GLP-1 is a peptide drug with glucose-dependent insulinotropic effects, thereby helping to avoid the potential risk of hypoglycemia associated with insulin use. However, its rapid plasma clearance and enzymatic degradation have hampered the possibility of its use as a real option for the treatment of diabetes. In order to overcome these difficulties and move toward its future clinical use, Chilkoti's group has developed fusion constructs between an ELR and GLP-1 in which the ELR plays an active role by allowing depot formation after injection and also acts as a long-circulating macromolecular carrier.[92] Thus, GLP-1-ELR fusions were demonstrated to be proteolytically more stable than the GLP-1 monomer, probably due to a steric effect of the ELR, which hinders access of proteases to their cleavage site. Moreover, in vivo studies conducted in a mouse model showed that (GLP-1)-ELR depot displays an extended temporal reduction of glucose levels by approximately 30% for 5 days. In contrast, both controls, namely, (GLP-1)-ELR monomer and GLP-1, resulted in a sharp and nonsustained drop in glucose levels.

Using a similar approach, a (GLP-1)-ELR depot was designed so that GLP-1 is released in a monomeric form (without an ELR).[93] In this design, GLP-1 oligomers are flanked by protease cleavage sites and fused to the ELR such that cleavage at these sites allows GLP-1 release from the depot. The resulting depot was termed a "protease operated depot" (POD). A single injection of GLP-1-POD was subsequently shown to reduce blood glucose levels in mice for up to 5 days.

As stated above, injection of a pharmaceutical device directly into the target tissue minimizes the side effects on healthy tissues as well as allowing complicated locations that are not easily accessible by systemic administration to be reached. A good example of a body location that is not easily reached is the joints, since their avascular nature complicates access via systemic administration. In this sense, intra-articular injection of ELR-depots has been explored as a feasible option to achieve controlled drug delivery in such locations. Thus, biodistribution of an ELR after injection into the knee joint compartment in a rat model demonstrated that the ability of the ELR to aggregate prolonged the half-life of the polymer by more than 25-fold when compared to a soluble nonaggregating ELR.[94]

A further step in the application of this kind of ELR-depot in the treatment of joint diseases, such as osteoarthritis, involved the construction of a fusion construct between an ELR and IL-1Ra (interleukin-1 receptor antagonist).[95] Although the interaction of IL-1Ra fused to the ELR moieties with IL-R was slower when compared to the free IL-1Ra, such an

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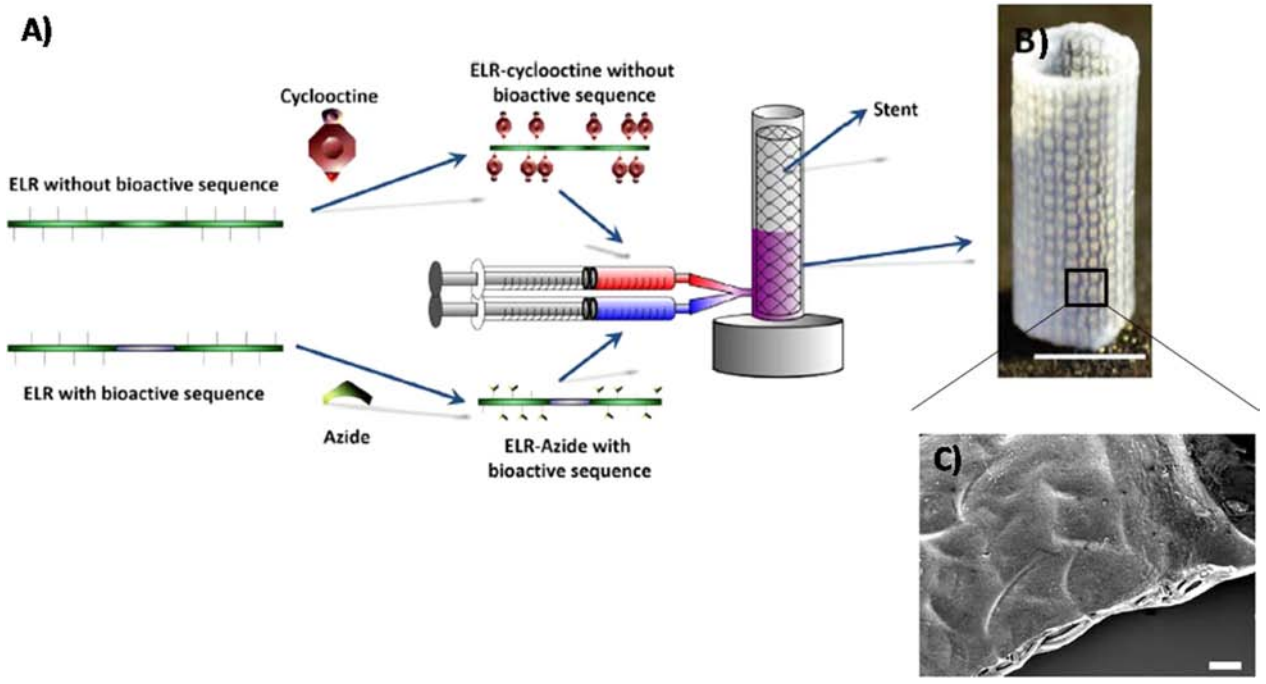

Figure 5. (A) Schematic representation of the molding procedure of Click-ELR-coated stents. (B) Warp-knitted nitinol stent coated with Click-ELR. (C) SEM image of a Click-ELR stent.[113]

approach represents an appropriate strategy for prolonging the presence of bioactive therapeutic agents following intra-articular delivery.

When immunosuppression treatments are required, the possibility of local delivery becomes a must to avoid systemic exposure. Thus, a tritium-radiolabeled ELR (depot and soluble) has been injected overlying the L5 dorsal root ganglion of rats, with the resulting biodistribution data clearly showing an enhanced half-life (7-fold longer) of ELR depot in the perineural space when compared to the soluble ELR along with a 14-fold reduction in systemic exposure.[96] This study opens the way to the use of ELR depots as effective devices for the release of immunomodulating therapeutic agents to treat local neuroinflammation. Another striking approach related to the use of ELR depots in neuroinflammation treatment involved the development of an injectable depot in which curcumin (a TNFα antagonist) was conjugated to the ELR via a degradable carbamate linkage.[97] Intramuscular injection of the resulting curcumin-ELR depot proximal to the sciatic nerve in mice resulted in a 5-fold higher level of the drug at 96 h as compared to the free drug while limiting systemic exposure (7-fold reduction).

Radiation therapy is a widely used strategy for the treatment of tumors. Motivated by the development of a radionuclide carrier that is able to prevent dissemination from the injection site, an ELR conjugated with iodine-131 depot has been constructed.[98] The effectiveness of this depot arises due to some of the particular characteristics of radionuclide therapy. For example, drug release from the ELR carrier is not necessary. Moreover, radionuclide emissions can kill tumor cells from a distance (e.g., the penetration distance of ^{131}I β-emission is 910 μm), thus meaning that no internalization by tumor cells is required. In this approach, although a delay in significant tumor growth was observed, complete regression still required some optimization in terms of ELR design, concentration, and infusion protocol. This optimization was subsequently achieved thanks to a meticulous and systematic study of the physical properties of the ELR and their influence on tumor retention and the concomitant translation into a therapeutic advantage.[99] Thus, the optimized ^{131}I-ELR depot delayed tumor growth in 100% of the tumors in two human xenografts (FaDu and PC-3) and cured more than 67% of tumor-bearing animals.

The characteristic hydrophobic associations of ELRs have been exploited in the aforementioned examples to achieve physically cross-linked depots or hydrogels. However, non-covalent bonding usually results in suboptimal mechanical properties. In order to overcome this issue, Chilkoti's group has developed hydrogels with properties intermediate between those of coacervates and chemical hydrogels by engineering the presence of cysteine residues along the ELR structure.[100] The resulting hydrogels display rapid gelation under mild oxidative conditions and their in vivo intratumoral administration to nude mice bearing human pharynx squamous xenografts showed enhanced tumor retention when compared to their control (ELR without cysteine), in addition to an ideal homogeneous distribution across the entire tumor.

One remarkable strategy for obtaining entirely physical and stable ELR-based hydrogels is to incorporate bioinspired peptide motifs other than elastin along the ELR backbone. One interesting example of bioinspired peptide motifs for physical cross-linking purposes is the use of silk fibroin domains. Such domains, the sequence of which follows patterns such as GAGAGS, are known to adopt an antiparallel β-sheet structure characterized by high stability and irreversibility. Such properties are maintained when GAGAGS motifs are engineering along an ELR molecule, giving rise to so-called SELRs (silk-elastin like recombinamers). Many SELR-based hydrogels have been reported in the literature,[101−104] and some of them have been successfully explored for drug-delivery purposes.[105−108] Indeed, the utility of SELRs has been explored both in the field of drug delivery and for the delivery of viral vectors. Specifically, an adenovirus containing both thymidine kinase-1 and

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luciferase genes has been incorporated into an SELR matrix (SELP 815K hydrogel) and the resulting system injected intratumorally into a nude mouse model of head and neck cancer.[109] Bioluminescence provided by the luciferase gene was used to determine gene transfection efficiency, duration of transgene expression, and biodistribution. It was clearly shown that administration within the SELP 815K hydrogel resulted in greater confinement of the therapeutic agent at the tumor site, with no evidence of any spreading to the liver, thereby contrasting with the results obtained for the saline/adenovirus treatment group, in which 50% of animals showed clear liver dissemination. With regard to anticancer efficacy, an up to 5-fold reduction in tumor volume was observed in the adenovirus-SELP 815K group when compared to the saline/adenovirus treatment group. In a further design step, an advanced version characterized by the presence of a matrix-metalloproteinase (MMP) sequence along the SELP 815K was constructed.[103] The MMP-sensitivity of these hydrogels suggests that they could potentially respond to local changes in these proteases when injected intratumorally.

In addition to the aforementioned approaches, in which the self-assembly behavior of elastin-based constructs is exploited to form depots and hydrogels, another approach for achieving and maintaining a three-dimensional ELR-based scaffold involves the inclusion of an amino acid that can subsequently be modified to contain chemical groups for covalent cross-linking.[110] Such strategies usually involve incorporation of the lysine residue "K" at the "X" position of some of the (VPGXG) pentapeptides present in the ELR. For example, ELRs have been chemically modified at their lysine amino acids to bear the reactive groups required for "click chemistry" reactions, namely, azide and cyclooctyne. Since the chemically reactive groups are incorporated into the ELR structure prior to the cross-linking reaction, there is no risk of the release of excess cross-linking reagents. Furthermore, subsequent covalent cross-linking takes place under mild physiological conditions and with short reaction times.[111,112] Such an approach would permit the homogeneous entrapment of drugs and biomolecules inside the hydrogel, while guaranteeing their biocompatibility. In a further step, the Click-ELR hydrogels reported previously[112] have been used to coat vascular stents (Figure 5),[113] thus paving the way to the next generation of stents with drug-release properties. Another approach for obtaining high selectivity involves taking advantage of the specificity achieved by enzymatic reactions, such as that catalyzed by transglutaminase.[114] For example, ELR hydrogels cross-linked via transglutaminase have been engineered so that exposure to the proteolytic activity derived from *P. aeruginose* and human polymorphonuclear leukocytes triggers the release of a model compound from the matrix.[115] The final goal of this kind of approach, in which the hydrogel is engineered to be sensitive to specific proteases, is to obtain a vehicle that can sense a chemical abnormality in damaged tissue and release a therapeutic agent in response to it. Thus, the well-established correlation between many relevant pathological conditions and elastolytic conditions could potentially allow the sustained release of drugs that ultimately interfere with the pathological process that triggered its release.

The above examples reflect the strong commitment of the scientific community to the development of novel and advanced drug-delivery depots and hydrogels that are able to meet specific therapeutic needs. Although it is clear that there have been several significant breakthroughs in this field, there is still a long way to go to achieve the ultimate goal, namely, to create devices that are able to act as the body does by detecting where, when, and how they should release their own biological compounds.

5. CONCLUSIONS

There is currently an increasing need for new pharmacological platforms that can release their therapeutic agents in a well-controlled spatiotemporal manner. Additionally, this goal must be accomplished in the complex environment generated by living tissues, bodily fluids, and/or even inside cells. As such, these delivery systems must be intrinsically complex as they need to address the multiple challenges that living systems will generate during their action. In this sense, ever more sophisticated materials are needed in order to achieve truly efficient and advanced drug-delivery systems. Among the many options currently being explored, protein-based polymers and, in particular, elastin-like recombinamers stand out due to the possibility of incorporating virtually any peptide-function present (or not) in any natural protein into their composition, thus making these materials a source of therapeutic agents in their own right. However, they may also be excellent carriers for delivering drugs to a designated target tissue or even intracellular location. Once again, the advantage of incorporating functional peptides that can help to protect the therapeutic agent and precisely deliver it to its final destination within their peptide sequence is a remarkable advantage arising from the use of these kinds of materials. The combination of biofunctionality and an ability to self-assemble, which can easily be combined and exploited in such protein-based materials, is unique. Additionally, as these materials are produced using a purely synthetic gene, genetic engineering approaches can applied in an essentially unrestricted manner to ensure that the final composition is only dictated by these engineering designs. All this, along with the other additional advantages described above, has already resulted in therapeutic platforms with markedly increased efficacy. However, since the potential of this approach is far from being fully explored, this is just the beginning, and the next few years will witness the development and clinical application of sophisticated ELR-based therapeutic platforms that will open up the way to therapeutic strategies that are currently beyond our reach.

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Notes

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REFERENCES

(1) Allen, T. M., and Cullis, P. R. (2004) Drug delivery systems: entering the mainstream. *Science 303*, 1818−1822.

DOI: 10.1021/acs.bioconjchem.5b00183
Bioconjugate Chem. 2015, 26, 1252−1265

(2) Langer, R. (1990) New methods of drug delivery. *Science 249*, 1527−33.
(3) Price, R., Poursaid, A., and Ghandehari, H. (2014) Controlled release from recombinant polymers. *J. Controlled Release 190*, 304−13.
(4) MacEwan, S. R., and Chilkoti, A. (2010) Elastin-like polypeptides: Biomedical applications of tunable biopolymers. *Biopolymers 94*, 60−77.
(5) MacEwan, S. R., and Chilkoti, A. (2014) Applications of elastin-like polypeptides in drug delivery. *J. Controlled Release 190*, 314−30.
(6) Rodriguez-Cabello, J. C., Gonzalez de Torre, I., and Pinedo, G. (2013) Elastin-like hydrogels and self-assembled nanostructures for drug delivery, in *Smart Materials for Drug Delivery: Volume 2*, pp 180−198, Chaper 19, The Royal Society of Chemistry.
(7) Girotti, A., Fernandez-Colino, A., Lopez, I. M., Rodriguez-Cabello, J. C., and Arias, F. J. (2011) Elastin-like recombinamers: biosynthetic strategies and biotechnological applications. *Biotechnol. J. 6*, 1174−86.
(8) Rodriguez-Cabello, J. C., Girotti, A., Ribeiro, A., and Arias, F. J. (2012) Synthesis of genetically engineered protein polymers (recombinamers) as an example of advanced self-assembled smart materials, in *Nanotechnology in Regenerative Medicine* (Navarro, M., and Planell, J. A., Eds.) pp 319, Humana Press.
(9) Arias, F. J., Santos, M., Fernández-Colino, A., Pinedo, G., and Girotti, A. (2014) Recent contributions of elastin-like recombinamers to biomedicine and nanotechnology. *Curr. Top. Med. Chem. 14*, 819−836.
(10) Rodriguez-Cabello, J. C., Pierna, M., Fernandez-Colino, A., Garcia-Arevalo, C., and Arias, F. J. (2011) Recombinamers: combining molecular complexity with diverse bioactivities for advanced biomedical and biotechnological applications. *Adv. Biochem. Eng./Biotechnol. 125*, 145−79.
(11) Urry, D. W. (1993) Molecular machines - how motion and other functions of living organisms can result from reversible chemical-changes. *Angew. Chem., Int. Ed. 32*, 819−841.
(12) Urry, D. W. (2006) *What sustains life? Consilient mechanisms for protein-based machines and materials*, Springer-Verlag, New York.
(13) Venkatachalam, C. M., and Urry, D. W. (1981) Development of a linear helical conformation from its cyclic correlate. *β*-Spiral model of the elastin poly(pentapeptide) (VPGVG)n. *Macromolecules 14*, 1225−1229.
(14) Urry, D. W. (1997) Physical chemistry of biological free energy transduction as demonstrated by elastic protein-based polymers. *J. Phys. Chem. B 101*, 11007−11028.
(15) Li, B., Alonso, D. O. V., and Daggett, V. (2001) The molecular basis for the inverse temperature transition of elastin. *J. Mol. Biol. 305*, 581−592.
(16) Stuart, M. A., Huck, W. T., Genzer, J., Muller, M., Ober, C., Stamm, M., Sukhorukov, G. B., Szleifer, I., Tsukruk, V. V., Urban, M., et al. (2010) Emerging applications of stimuli-responsive polymer materials. *Nat. Mater. 9*, 101−13.
(17) Nikolov, Z. L., and Woodard, S. L. (2004) Downstream processing of recombinant proteins from transgenic feedstock. *Curr. Opin. Biotechnol. 15*, 479−86.
(18) Meyer, D. E., and Chilkoti, A. (1999) Purification of recombinant proteins by fusion with thermally-responsive polypeptides. *Nat. Biotechnol. 17*, 1112−5.
(19) Hassouneh, W., Christensen, T., and Chilkoti, A. (2010) Elastin-like polypeptides as a purification tag for recombinant proteins, in *Current Protocols in Protein Science*, Chapter 6, Unit 6 11, Wiley.
(20) Trabbic-Carlson, K., Liu, L., Kim, B., and 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.
(21) Osicka, R., Prochazkova, K., Sulc, M., Linhartova, I., Havlicek, V., and Sebo, P. (2004) A novel "clip-and-link" activity of repeat in toxin (RTX) proteins from gram-negative pathogens. Covalent protein cross-linking by an Asp-Lys isopeptide bond upon calcium-dependent processing at an Asp-Pro bond. *J. Biol. Chem. 279*, 24944−56.
(22) Liu, W. J., Wu, Q., Xu, B., Zhang, X. Y., Xia, X. L., and Sun, H. C. (2014) Single-step purification of recombinant proteins using elastin-like peptide-mediated inverse transition cycling and self-processing module from Neisseria meningitides FrpC. *Protein Expression Purif. 98*, 18−24.
(23) Perler, F. B., Davis, E. O., Dean, G. E., Gimble, F. S., Jack, W. E., Neff, N., Noren, C. J., Thorner, J., and Belfort, M. (1994) Protein splicing elements: inteins and exteins–a definition of terms and recommended nomenclature. *Nucleic Acids Res. 22*, 1125−7.
(24) Wood, D. W., Wu, W., Belfort, G., Derbyshire, V., and Belfort, M. (1999) A genetic system yields self-cleaving inteins for bioseparations. *Nat. Biotechnol. 17*, 889−92.
(25) Banki, M. R., Feng, L., and Wood, D. W. (2005) Simple bioseparations using self-cleaving elastin-like polypeptide tags. *Nat. Methods 2*, 659−61.
(26) Fong, B. A., and Wood, D. W. (2010) Expression and purification of ELP-intein-tagged target proteins in high cell density E. coli fermentation. *Microb. Cell Fact. 9*, 77.
(27) Fong, B. A., Gillies, A. R., Ghazi, I., LeRoy, G., Lee, K. C., Westblade, L. F., and Wood, D. W. (2010) Purification of Escherichia coli RNA polymerase using a self-cleaving elastin-like polypeptide tag. *Protein Sci. 19*, 1243−52.
(28) Park, H.-B., Pyo, S.-H., Hong, S.-S., and Kim, J.-H. (2001) Optimization of the hydroxylamine cleavage of an expressed fusion protein to produce a recombinant antimicrobial peptide. *Biotechnol. Lett. 23*, 637−641.
(29) Hu, F., Ke, T., Li, X., Mao, P. H., Jin, X., Hui, F. L., Ma, X. D., and Ma, L. X. (2010) Expression and purification of an antimicrobial peptide by fusion with elastin-like polypeptides in Escherichia coli. *Appl. Biochem. Biotechnol. 160*, 2377−87.
(30) Hara, S., and Yamakawa, M. (1996) Production in Escherichia of moricin, a novel type antibacterial peptide from the silkworm, Bombyx mori. *Biochem. Biophys. Res. Commun. 220*, 664−9.
(31) Gerashchenko, O. L., Zhuravel, E. V., Skachkova, O. V., Khranovska, N. N., Filonenko, V. V., Pogrebnoy, P. V., and Soldatkina, M. A. (2013) Biologic activities of recombinant human-beta-defensin-4 toward cultured human cancer cells. *Exp. Oncol. 35*, 76−82.
(32) Shen, Y., Ai, H. X., Song, R., Liang, Z. N., Li, J. F., and Zhang, S. Q. (2010) Expression and purification of moricin CM4 and human beta-defensins 4 in Escherichia coli using a new technology. *Microbiol. Res. 165*, 713−8.
(33) Lim, D. W., Trabbic-Carlson, K., Mackay, J. A., and Chilkoti, A. (2007) Improved non-chromatographic purification of a recombinant protein by cationic elastin-like polypeptides. *Biomacromolecules 8*, 1417−24.
(34) Xu, X., Jin, F., Yu, X., Ji, S., Wang, J., Cheng, H., Wang, C., and Zhang, W. (2007) Expression and purification of a recombinant antibacterial peptide, cecropin, from Escherichia coli. *Protein Expression Purif. 53*, 293−301.
(35) Yang, K., Su, Y., Li, J., Sun, J., and Yang, Y. (2012) Expression and purification of the antimicrobial peptide cecropin AD by fusion with cationic elastin-like polypeptides. *Protein Expression Purif. 85*, 200−3.
(36) Bellucci, J. J., Amiram, M., Bhattacharyya, J., McCafferty, D., and 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.
(37) Dreher, M. R., Raucher, D., Balu, N., Michael Colvin, O., Ludeman, S. M., and Chilkoti, A. (2003) Evaluation of an elastin-like polypeptide-doxorubicin conjugate for cancer therapy. *J. Controlled Release 91*, 31−43.
(38) Furgeson, D. Y., Dreher, M. R., and Chilkoti, A. (2006) Structural optimization of a "smart" doxorubicin-polypeptide conjugate for thermally targeted delivery to solid tumors. *J. Controlled Release 110*, 362−9.
(39) Bidwell, G. L., 3rd, Davis, A. N., Fokt, I., Priebe, W., and Raucher, D. (2007) A thermally targeted elastin-like polypeptide-

DOI: 10.1021/acs.bioconjchem.5b00183
Bioconjugate Chem. 2015, 26, 1252−1265

doxorubicin conjugate overcomes drug resistance. *Invest. New Drugs 25*, 313−26.
(40) Meyer, D. E., Kong, G. A., Dewhirst, M. W., Zalutsky, M. R., and Chilkoti, A. (2001) Targeting a genetically engineered elastin-like polypeptide to solid tumors by local hyperthermia. *Cancer Res. 61*, 1548−54.
(41) Raucher, D., and Chilkoti, A. (2001) Enhanced uptake of a thermally responsive polypeptide by tumor cells in response to its hyperthermia-mediated phase transition. *Cancer Res. 61*, 7163−70.
(42) Bidwell, G. L., 3rd, Fokt, I., Priebe, W., and Raucher, D. (2007) Development of elastin-like polypeptide for thermally targeted delivery of doxorubicin. *Biochem. Pharmacol. 73*, 620−31.
(43) Moktan, S., Perkins, E., Kratz, F., and Raucher, D. (2012) Thermal targeting of an acid-sensitive doxorubicin conjugate of elastin-like polypeptide enhances the therapeutic efficacy compared with the parent compound in vivo. *Mol. Cancer Ther. 11*, 1547−56.
(44) Moktan, S., Ryppa, C., Kratz, F., and Raucher, D. (2012) A thermally responsive biopolymer conjugated to an acid-sensitive derivative of paclitaxel stabilizes microtubules, arrests cell cycle, and induces apoptosis. *Invest. New Drugs 30*, 236−48.
(45) Conley, A. J., Joensuu, J. J., Jevnikar, A. M., Menassa, R., and Brandle, J. E. (2009) Optimization of elastin-like polypeptide fusions for expression and purification of recombinant proteins in plants. *Biotechnol. Bioeng. 103*, 562−73.
(46) Patel, J., Zhu, H., Menassa, R., Gyenis, L., Richman, A., and Brandle, J. (2007) Elastin-like polypeptide fusions enhance the accumulation of recombinant proteins in tobacco leaves. *Transgenic Res. 16*, 239−49.
(47) Conrad, U., Plagmann, I., Malchow, S., Sack, M., Floss, D. M., Kruglov, A. A., Nedospasov, S. A., Rose-John, S., and Scheller, J. (2011) ELPylated anti-human TNF therapeutic single-domain antibodies for prevention of lethal septic shock. *Plant Biotechnol. J. 9*, 22−31.
(48) Kaldis, A., Ahmad, A., Reid, A., McGarvey, B., Brandle, J., Ma, S., Jevnikar, A., Kohalmi, S. E., and Menassa, R. (2013) High-level production of human interleukin-10 fusions in tobacco cell suspension cultures. *Plant Biotechnol. J. 11*, 535−45.
(49) Floss, D. M., Sack, M., Arcalis, E., Stadlmann, J., Quendler, H., Rademacher, T., Stoger, E., Scheller, J., Fischer, R., and Conrad, U. (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.
(50) Sarangthem, V., Cho, E. A., Bae, S. M., Singh, T. D., Kim, S. J., Kim, S., Jeon, W. B., Lee, B. H., and Park, R. W. (2013) Construction and application of elastin like polypeptide containing IL-4 receptor targeting peptide. *PLoS One 8*, e81891.
(51) Wang, W., Jashnani, A., Aluri, S. R., Gustafson, J. A., Hsueh, P. Y., Yarber, F., McKown, R. L., Laurie, G. W., Hamm-Alvarez, S. F., and MacKay, J. A. (2015) A thermo-responsive protein treatment for dry eyes. *J. Controlled Release 199*, 156−67.
(52) Bidwell, G. L., 3rd, and Raucher, D. (2005) Application of thermally responsive polypeptides directed against c-Myc transcriptional function for cancer therapy. *Mol. Cancer Ther. 4*, 1076−85.
(53) Bidwell, G. L., 3rd, Davis, A. N., and Raucher, D. (2009) Targeting a c-Myc inhibitory polypeptide to specific intracellular compartments using cell penetrating peptides. *J. Controlled Release 135*, 2−10.
(54) Bidwell, G. L., 3rd, Perkins, E., Hughes, J., Khan, M., James, J. R., and Raucher, D. (2013) Thermally targeted delivery of a c-Myc inhibitory polypeptide inhibits tumor progression and extends survival in a rat glioma model. *PLoS One 8*, e55104.
(55) Ryu, J. S., and Raucher, D. (2014) Elastin-like polypeptides: the influence of its molecular weight on local hyperthermia-induced tumor accumulation. *Eur. J. Pharm. Biopharm. 88*, 382−9.
(56) Massodi, I., Thomas, E., and Raucher, D. (2009) Application of thermally responsive elastin-like polypeptide fused to a lactoferrin-derived peptide for treatment of pancreatic cancer. *Molecules 14*, 1999−2015.
(57) Massodi, I., Moktan, S., Rawat, A., Bidwell, G. L., 3rd, and Raucher, D. (2010) Inhibition of ovarian cancer cell proliferation by a cell cycle inhibitory peptide fused to a thermally responsive polypeptide carrier. *Int. J. Cancer 126*, 533−44.
(58) Ryu, J. S., and Raucher, D. (2014) Anti-tumor efficacy of a therapeutic peptide based on thermo-responsive elastin-like polypeptide in combination with gemcitabine. *Cancer Lett. (N. Y., NY, U. S.) 348*, 177−84.
(59) Pinedo-Martín, G., Castro, E., Martín, L., Alonso, M., and Rodríguez-Cabello, J. C. (2014) Effect of surfactants on the self-assembly of a model elastin-like block corecombinamer: from micelles to an aqueous two-phase system. *Langmuir 30*, 3432−3440.
(60) Martin, L., Castro, E., Ribeiro, A., Alonso, M., and Rodriguez-Cabello, J. C. (2012) Temperature-triggered self-assembly of elastin-like block co-recombinamers:the controlled formation of micelles and vesicles in an aqueous medium. *Biomacromolecules 13*, 293−8.
(61) Wright, E. R., and Conticello, V. P. (2002) Self-assembly of block copolymers derived from elastin-mimetic polypeptide sequences. *Adv. Drug Delivery Rev. 54*, 1057−73.
(62) Rodríguez-Cabello, J. C., Martín, L., Alonso, M., Arias, F. J., and Testera, A. M. (2009) Recombinamers" as advanced materials for the post-oil age. *Polymer 50*, 5159−5169.
(63) Nettles, D. L., Chilkoti, A., and Setton, L. A. (2010) Applications of elastin-like polypeptides in tissue engineering. *Adv. Drug Delivery Rev. 62*, 1479−1485.
(64) Dreher, M. R., and Chilkoti, A. (2007) Toward a systems engineering approach to cancer drug delivery. *J. Natl. Cancer Inst. 99*, 983−5.
(65) McDaniel, J. R., Bhattacharyya, J., Vargo, K. B., Hassouneh, W., Hammer, D. A., and Chilkoti, A. (2013) Self-assembly of thermally responsive nanoparticles of a genetically encoded peptide polymer by drug conjugation. *Angew. Chem., Int. Ed. 52*, 1683−1687.
(66) MacKay, J. A., Chen, M., McDaniel, J. R., Liu, W., Simnick, A. J., and Chilkoti, A. (2009) Self-assembling chimeric polypeptide-doxorubicin conjugate nanoparticles that abolish tumours after a single injection. *Nat. Mater. 8*, 993−9.
(67) Piña, M. J., Alex, S. M., Arias, F. J., Santos, M., Rodriguez-Cabello, J. C., Ramesan, R. M., and Sharma, C. P. (2015) Elastin-like recombinamers with acquired functionalities for gene-delivery applications. *J. Biomed. Mater. Res., Part A* [Online Early Access] DOI: 10.1002/jbm.a.35455, March 16, 2015.
(68) Koria, P., Yagi, H., Kitagawa, Y., Megeed, Z., Nahmias, Y., Sheridan, R., and Yarmush, M. L. (2011) Self-assembling elastin-like peptides growth factor chimeric nanoparticles for the treatment of chronic wounds. *Proc. Natl. Acad. Sci. U. S. A. 108*, 1034−9.
(69) Bessa, P. C., Machado, R., Nürnberger, S., Dopler, D., Banerjee, A., Cunha, A. M., Rodríguez-Cabello, J. C., Redl, H., van Griensven, M., Reis, R. L., et al. (2010) Thermoresponsive self-assembled elastin-based nanoparticles for delivery of BMPs. *J. Controlled Release 142*, 312−318.
(70) Mackay, J. A., Callahan, D. J., Fitzgerald, K. N., and Chilkoti, A. (2010) Quantitative model of the phase behavior of recombinant pH-responsive elastin-like polypeptides. *Biomacromolecules 11*, 2873−9.
(71) Dreher, M. R., Simnick, A. J., Fischer, K., Smith, R. J., Patel, A., Schmidt, M., and Chilkoti, A. (2008) Temperature triggered self-assembly of polypeptides into multivalent spherical micelles. *J. Am. Chem. Soc. 130*, 687−94.
(72) Shi, P., Aluri, S., Lin, Y. A., Shah, M., Edman, M., Dhandhukia, J., Cui, H., and MacKay, J. A. (2013) Elastin-based protein polymer nanoparticles carrying drug at both corona and core suppress tumor growth in vivo. *J. Controlled Release 171*, 330−8.
(73) Sun, G., Hsueh, P. Y., Janib, S. M., Hamm-Alvarez, S., and MacKay, J. A. (2011) Design and cellular internalization of genetically engineered polypeptide nanoparticles displaying adenovirus knob domain. *J. Controlled Release 155*, 218−26.
(74) Na, K., Jung, J., Lee, J., and Hyun, J. (2010) Thermoresponsive pore structure of biopolymer microspheres for a smart drug carrier. *Langmuir 26*, 11165−9.

DOI: 10.1021/acs.bioconjchem.5b00183
Bioconjugate Chem. 2015, 26, 1252−1265

(75) Cheng, J., Park, M., Lim, D., and Hyun, J. (2013) Polypeptide microgel capsules as drug carriers. *Macromol. Res. 21*, 1163−1166.
(76) Costa, R. R., Castro, E., Arias, F. J., Rodríguez-Cabello, J. C., and Mano, J. F. (2013) Multifunctional compartmentalized capsules with a hierarchical organization from the nano to the macro scales. *Biomacromolecules 14*, 2403−2410.
(77) Costa, R. R., Girotti, A., Santos, M., Arias, F. J., Mano, J. F., and Rodriguez-Cabello, J. C. (2014) Cellular uptake of multilayered capsules produced with natural and genetically engineered biomimetic macromolecules. *Acta Biomater. 10*, 2653−62.
(78) Chen, T.-H., Bae, Y., and Furgeson, D. (2008) Intelligent biosynthetic nanobiomaterials (IBNs) for hyperthermic gene delivery. *Pharm. Res. 25*, 683−691.
(79) Garcia-Arevalo, C., Bermejo-Martin, J. F., Rico, L., Iglesias, V., Martin, L., Rodriguez-Cabello, J. C., and Arias, F. J. (2013) Immunomodulatory nanoparticles from elastin-like recombinamers: single-molecules for tuberculosis vaccine development. *Mol. Pharmaceutics 10*, 586−97.
(80) Falk, M. H., and Issels, R. D. (2001) Hyperthermia in oncology. *Int. J. Hyperthermia 17*, 1−18.
(81) Simnick, A. J., Valencia, C. A., Liu, R., and 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.
(82) Hassouneh, W., Fischer, K., MacEwan, S. R., Branscheid, R., Fu, C. L., Liu, R., Schmidt, M., and Chilkoti, A. (2012) Unexpected multivalent display of proteins by temperature triggered self-assembly of elastin-like polypeptide block copolymers. *Biomacromolecules 13*, 1598−605.
(83) MacEwan, S. R., and Chilkoti, A. (2014) Controlled apoptosis by a thermally toggled nanoscale amplifier of cellular uptake. *Nano Lett. 14*, 2058−64.
(84) MacEwan, S. R., and Chilkoti, A. (2012) Digital switching of local arginine density in a genetically encoded self-assembled polypeptide nanoparticle controls cellular uptake. *Nano Lett. 12*, 3322−3328.
(85) Hoare, T. R., and Kohane, D. S. (2008) Hydrogels in drug delivery: Progress and challenges. *Polymer 49*, 1993−2007.
(86) Altunbas, A., and Pochan, D. (2012) Peptide-Based and Polypeptide-Based Hydrogels for Drug Delivery and Tissue Engineering, in *Peptide-Based Materials* (Deming, T., Ed.) pp 135−167, Springer, Berlin.
(87) Yan, C., and Pochan, D. J. (2010) Rheological properties of peptide-based hydrogels for biomedical and other applications. *Chem. Soc. Rev. 39*, 3528−3540.
(88) Evans, C. H., Kraus, V. B., and Setton, L. A. (2014) Progress in intra-articular therapy. *Nat. Rev. Rheumatol. 10*, 11−22.
(89) Floss, D. M., Schallau, K., Rose-John, S., Conrad, U., and Scheller, J. (2010) Elastin-like polypeptides revolutionize recombinant protein expression and their biomedical application. *Trends Biotechnol. 28*, 37−45.
(90) Hassouneh, W., MacEwan, S. R., and Chilkoti, A. (2012) Fusions of elastin-like polypeptides to pharmaceutical proteins. *Methods Enzymol. 502*, 215−37.
(91) Meyer, D. E., and Chilkoti, A. (2002) Protein purification by inverse transition cycling, in *Protein-Protein Interactions: A Molecular Cloning Manual* (Golemis, E., and Adams, P. D., Eds.) pp 938, Cold Spring Harbor Laboratory Press.
(92) Amiram, M., Luginbuhl, K. M., Li, X., Feinglos, M. N., and Chilkoti, A. (2013) A depot-forming glucagon-like peptide-1 fusion protein reduces blood glucose for five days with a single injection. *J. Controlled Release 172*, 144−51.
(93) Amiram, M., Luginbuhl, K. M., Li, X., Feinglos, M. N., and Chilkoti, A. (2013) Injectable protease-operated depots of glucagon-like peptide-1 provide extended and tunable glucose control. *Proc. Natl. Acad. Sci. U. S. A. 110*, 2792−7.
(94) Betre, H., Liu, W., Zalutsky, M. R., Chilkoti, A., Kraus, V. B., and Setton, L. A. (2006) A thermally responsive biopolymer for intra-articular drug delivery. *J. Controlled Release 115*, 175−82.
(95) Shamji, M. F., Betre, H., Kraus, V. B., Chen, J., Chilkoti, A., Pichika, R., Masuda, K., and Setton, L. A. (2007) Development and characterization of a fusion protein between thermally responsive elastin-like polypeptide and interleukin-1 receptor antagonist: sustained release of a local antiinflammatory therapeutic. *Arthritis Rheum. 56*, 3650−61.
(96) Shamji, M. F., Whitlatch, L., Friedman, A. H., Richardson, W. J., Chilkoti, A., and Setton, L. A. (2008) An injectable and in situ-gelling biopolymer for sustained drug release following perineural administration. *Spine 33*, 748−54.
(97) Sinclair, S. M., Bhattacharyya, J., McDaniel, J. R., Gooden, D. M., Gopalaswamy, R., Chilkoti, A., and Setton, L. A. (2013) A genetically engineered thermally responsive sustained release curcumin depot to treat neuroinflammation. *J. Controlled Release 171*, 38−47.
(98) Liu, W., MacKay, J. A., Dreher, M. R., Chen, M., McDaniel, J. R., Simnick, A. J., Callahan, D. J., Zalutsky, M. R., and Chilkoti, A. (2010) Injectable intratumoral depot of thermally responsive polypeptide-radionuclide conjugates delays tumor progression in a mouse model. *J. Controlled Release 144*, 2−9.
(99) Liu, W., McDaniel, J., Li, X., Asai, D., Quiroz, F. G., Schaal, J., Park, J. S., Zalutsky, M., and Chilkoti, A. (2012) Brachytherapy using injectable seeds that are self-assembled from genetically encoded polypeptides in situ. *Cancer Res. 72*, 5956−65.
(100) Asai, D., Xu, D., Liu, W., Garcia Quiroz, F., Callahan, D. J., Zalutsky, M. R., Craig, S. L., and Chilkoti, A. (2012) Protein polymer hydrogels by in situ, rapid and reversible self-gelation. *Biomaterials 33*, 5451−8.
(101) Fernandez-Colino, A., Arias, F. J., Alonso, M., and Rodriguez-Cabello, J. C. (2014) Self-organized ECM-mimetic model based on an amphiphilic multiblock silk-elastin-like corecombinamer with a concomitant dual physical gelation process. *Biomacromolecules 15*, 3781−93.
(102) Xia, X. X., Xu, Q., Hu, X., Qin, G., and Kaplan, D. L. (2011) Tunable self-assembly of genetically engineered silk–elastin-like protein polymers. *Biomacromolecules 12*, 3844−50.
(103) Gustafson, J. A., Price, R. A., Frandsen, J., Henak, C. R., Cappello, J., and Ghandehari, H. (2013) Synthesis and characterization of a matrix-metalloproteinase responsive silk-elastinlike protein polymer. *Biomacromolecules 14*, 618−25.
(104) Hu, X., Wang, X., Rnjak, J., Weiss, A. S., and Kaplan, D. L. (2010) Biomaterials derived from silk-tropoelastin protein systems. *Biomaterials 31*, 8121−31.
(105) Cappello, J., Crissman, J. W., Crissman, M., Ferrari, F. A., Textor, G., Wallis, O., Whitledge, J. R., Zhou, X., Burman, D., Aukerman, L., et al. (1998) In-situ self-assembling protein polymer gel systems for administration, delivery, and release of drugs. *J. Controlled Release 53*, 105−17.
(106) Megeed, Z., Cappello, J., and Ghandehari, H. (2002) Genetically engineered silk-elastinlike protein polymers for controlled drug delivery. *Adv. Drug Delivery Rev. 54*, 1075−1091.
(107) Huang, W., Rollett, A., and Kaplan, D. L. (2014) Silk-elastin-like protein biomaterials for the controlled delivery of therapeutics. *Expert Opin. Drug Delivery*, 1−13.
(108) Yucel, T., Lovett, M. L., and Kaplan, D. L. (2014) Silk-based biomaterials for sustained drug delivery. *J. Controlled Release 190*, 381−97.
(109) Greish, K., Frandsen, J., Scharff, S., Gustafson, J., Cappello, J., Li, D., O'Malley, B. W., Jr., and Ghandehari, H. (2010) Silk-elastinlike protein polymers improve the efficacy of adenovirus thymidine kinase enzyme prodrug therapy of head and neck tumors. *J. Gene Med. 12*, 572−9.
(110) Hennink, W. E., and van Nostrum, C. F. (2002) Novel crosslinking methods to design hydrogels. *Adv. Drug Delivery Rev. 54*, 13−36.
(111) Testera, A. M., Girotti, A., de Torre, I. G., Quintanilla, L., Santos, M., Alonso, M., and Rodriguez-Cabello, J. C. (2015) Biocompatible elastin-like click gels: design, synthesis and characterization. *J. Mater. Sci.: Mater. Med. 26*, 105.

DOI: 10.1021/acs.bioconjchem.5b00183
Bioconjugate Chem. 2015, 26, 1252−1265

(112) Gonzalez de Torre, I., Santos, M., Quintanilla, L., Testera, A., Alonso, M., and Rodriguez Cabello, J. C. (2014) Elastin-like recombinamer catalyst-free click gels: characterization of poroelastic and intrinsic viscoelastic properties. *Acta Biomater. 10*, 2495−505.
(113) de Torre, I. G., Wolf, F., Santos, M., Rongen, L., Alonso, M., Jockenhoevel, S., Rodriguez-Cabello, J. C., and Mela, P. (2015) Elastin-like recombinamer-covered stents: Towards a fully biocompatible and non-thrombogenic device for cardiovascular diseases. *Acta Biomater. 12*, 146−55.
(114) Hu, B.-H., and Messersmith, P. B. (2003) Rational design of transglutaminase substrate peptides for rapid enzymatic formation of hydrogels. *J. Am. Chem. Soc. 125*, 14298−14299.
(115) Bandiera, A., Markulin, A., Corich, L., Vita, F., and Borelli, V. (2014) Stimuli-induced release of compounds from elastin biomimetic matrix. *Biomacromolecules 15*, 416−22.

DOI: 10.1021/acs.bioconjchem.5b00183
Bioconjugate Chem. 2015, 26, 1252−1265