Elastin-like polypeptides in drug delivery

Contents

Keywords: Elastin-like recombinamers (ELRs), drug delivery, depots, nanoparticles, smart materials, self-assembling, chemotherapeutic agents.

Abstract

The use of recombinant elastin-like materials, or elastin-like recombinamers (ELRs), in drug-delivery applications is reviewed in this work. Although ELRs were initially used in similar ways to other, more conventional kinds of polymeric carriers, their unique properties soon gave rise to systems of unparalleled functionality and efficiency, with the stimuli responsiveness of ELRs and their ability to self-assemble readily allowing the creation of advanced systems. However, their recombinant nature is likely the most

important factor that has driven the current breakthrough properties of ELR-based delivery systems. Recombinant technology allows an unprecedented degree of complexity in macromolecular design and synthesis. In addition, recombinant materials easily incorporate any functional domain present in natural proteins. Therefore, ELR-based delivery systems can exhibit complex interactions with both their drug load and the tissues and cells towards which this load is directed. Selected examples, ranging from highly functional nanocarriers to macrodepots, will be presented.

1. Introduction

From a classical point of view, controlled drug-delivery systems have well-defined goals, including the maintenance of adequate drug levels for sufficiently prolonged periods of time. To that end, drug-delivery systems act by increasing drug solubility and availability as well as modifying the pharmacokinetics of the drug to achieve its sustained and continuous presence. The materials and formulations used for this initial principle have been extensively reviewed elsewhere [1].

The role of the drug-delivery system can, however, be more ambitious, with properties such as targeting being pursued in even the very earliest designs. In this sense, the initial goal of maintaining adequate drug levels is combined with spatial control of action. In this approach, the drug-delivery system should be able to find the sites where the drug is needed and release its cargo at these sites, thereby avoiding the exposure of healthy tissues and regions to the drug. Although this property of targeting is always relevant as a general ideal behaviour of any drug-delivery system, it is particularly relevant in areas such as anticancer drugs as anticancer therapies are always accompanied by severe side effects that can be substantially reduced by using such targeted drug-delivery systems.

Evidently, the possibilities for improving the efficiency of drug-delivery systems do not end at this level and, once again, novel approaches continue to be developed. Some examples of more sophisticated drug-delivery systems include intracellular drug delivery or spatial-temporal control of the delivery of combined drugs [2], or tailored devices suitable for a particular drug and disease combination. The ideal system is one that combines sensing with delivery in the sense that the system is inactive until a certain pathological condition is detected, at which point the drug is released to correct this adverse condition [3].

The evolutionary progress in the development of advanced drug-delivery systems has led, in turn, to the development of advanced materials. As the complex behaviour of such systems can is only based on the complex functionality of the materials used to build them, there is always a direct relationship between the complexity of the system and the complexity of the materials used to create the system. In essence, our ability to create complex and sophisticated materials limits the degree of complexity and functionality that can be achieved when developing such advanced drug-delivery systems. However, generally speaking, the synthesis of complex materials is time- and resource-consuming. Chemical synthesis has an important drawback, namely that the production costs of a synthetic material increase exponentially with its molecular complexity. Nowadays there is only one exception in which increasing complexity is not linked to increasing production costs – the recombinant materials also known as "recombinamers" [4], which are based on synthetic genes. Taking advantage of the current development of genetic engineering, it is now possible, for the first time, to create a synthetic gene with a composition that is controlled at the single base-pair level. The gene sequence is therefore essentially unlimited as it is not restricted to those genes related to natural proteins; although, evidently, natural proteins are an immense source of inspiration in the search for functional domains. Finally, these genes can be inserted into the genome of a producing cell, typically a microorganism, for expression, and this modified microorganism becomes a nearly infinite and cheap source of the desired material. Although creation of the gene may require an initial investment, this work only has to be performed once. After that, cell-based production rapidly compensates these initial costs related to gene construction. As such, recombinamers are proteinaceous materials based on a synthetic gene with a well-defined and -engineered design. They usually have a high molecular weight, thus meaning that they are truly macromolecules, and can be particularly complex, much more complex than any other macromolecule produced by chemical synthesis. In addition, the composition of these systems is strictly controlled, thus meaning that complexity and control form a binomial that can expand functionality to unseen levels. Their useful properties have motivated their use in a wide variety of materials and biological applications and, indeed, they show a huge potential as breakthrough materials in the development of advanced drug-delivery systems. In this regard, in addition to elastin-like recombinamers, several classes of recombinant polymers, such as silk-like, and silk-elastin-, collagen- and resilin-like recombinamers have been used for controlled drug delivery [5-8]. For

example, given the benefits of combining semi-crystalline silk-blocks and elastomeric elastin-blocks, SELRs possess multi-stimuli-responsive properties and tunability, thereby becoming promising candidates for the targeted delivery of anticancer drugs and controlled gene release [9]. Similarly, resilin-like-recombinamer-based nanoparticles could also find potential uses as responsive components in drug-delivery applications. Thus, Li *et al.* have demonstrated that the transition temperature and sizes of RLR-based nanoparticles can be modulated by varying the polypeptide concentration, salt identity, ionic strength, pH, and denaturing agents [10]. Additionally, thermoresponsive self-assembly of well-defined nanovesicles from a collagen-like peptide has also been demonstrated [11].

Natural elastin, which is one of the most abundant fibrous proteins in the vertebrate extracellular matrix, provides elasticity to flexible tissues and is the prevailing constituent of mature elastic fibers [12]. In addition to performing a mechanical and structural role, elastin acts as a signalling molecule to modulate cell-matrix interactions. Along the elastin sequence in fact have been identified motifs that control the cell behaviour as well as the "matrikine" hexapeptide VGVAPG [13], the consensus sequence GXXVP that interact with the elastin/laminin receptor [14], or the integrin-mediate cell adhesion motif GRKRK [15]. These bioactive motifs are able to induce and regulate, the cell adhesion, proliferation or differentiation and possess chemotactic properties on keratinocytes, fibroblasts, neutrophils and monocytes in addition to drive the extracellular matrix remodelling[16].

ELR compositions are inspired by the natural elastin precursor tropoelastin, especially the recurrent tetra- and pentapeptides found in its composition of the elastomeric domains [17]. Of these, the most widely used repetitive peptide in ELRs is the pentapeptide VPGXG, and some equivalent variations, where X represents any amino acid except L-proline. The choice of guest amino acid in the pentapeptide sequence can dramatically alter the physicochemical properties of the final recombinamer, therefore balancing the composition depending on the choice of this amino acid, depending on the final properties desired, is a key parameter when designing the initial basic composition of the ELR. Although detailed information regarding the dependence of these properties on the choice of the guest amino acid can be found elsewhere [17], in short, and in addition to the general advantages inherent to recombinant materials described above,

ELRs are stimuli-responsive materials. The mechanism of self-assembly of ELRs and their sensitivity to different stimuli has been described extensively in the literature. Their most striking property is perhaps the acute smart nature of these polymers. This smart nature is based upon a molecular transition of the polymer chain that takes place in the presence of water. This transition, known as the inverse temperature transition ("ITT") and first described for ELPs, has become the key issue in the development of peptide-based elastin. Indeed, all functional ELRs exhibit this reversible phase transition behavior [18]. In aqueous solution, and below a certain critical temperature (T_t), the free polymer chains remain as disordered, random coils in solution [19] that are fully hydrated, mainly by hydrophobic hydration. This hydration is characterized by the existence of ordered clathrate-like water structures surrounding the apolar moieties of the polymer[20-22] with a structure resembling that described for crystalline gas hydrates[22, 23]. In contrast, above T_t, poly(GVGVP) folds hydrophobically and assembles to form a phase-separated state containing 63% water and 37% polymer by weight[24] in which the polymer chains adopt a dynamic, regular, non-random structure known as a β-spiral, involving one type II β-turn per pentamer, and stabilized by intra-spiral inter-turn and inter-spiral hydrophobic contacts[18]. This is the product of the ITT. In this folded and associated state, the chain loses essentially all of the ordered water structures resulting from hydrophobic hydration [20]. During the initial stages of polymer dehydration, hydrophobic association of β-spirals takes on fibrillar form that grows into a particle several hundred nanometres in size before settling into the visible phase-separated state [18, 25]. This folding is completely reversible on lowering the sample temperature below T_t [18]. The ITT, and its associated T_t, is in many respects similar to a lower critical solution temperature (LCST) and many studies in this field have considered T_t and LCST to be equivalent even though the existence of a regularly folded structure—the beta spiral—above T_t differentiates the behaviour of an ELR from many other macromolecules exhibiting an LCST.

In ELRs, T_t depends on the mean polarity of the polymer, increasing as the hydrophobicity decreases. This is the origin of the so-called "ΔT_t mechanism" [18] and "amplified ΔT_t mechanism" [26]; i.e., if a chemical group that can be present in two different states of polarity is present in the polymer chain, and these states are reversibly convertible by the action of an external stimulus, the polymer will show two different T_t values. This change in T_t ("ΔT_t") opens a working temperature window in which the polymer isothermally and reversibly switches between the folded and unfolded states

following changes in the environmental stimulus. These ΔT_t mechanisms have been exploited to obtain a large number of smart elastin-like derivatives [18, 26-28]. In this sense, certain guest amino acids, such as glutamic/aspartic acid, lysine and others, confer pH sensitivity, a mechanism that is also exploited in the following model pH-responsive polymer. The γ-carboxylic function of the glutamic acid (E) residue in the ELR $[(VPGVG)_2\text{-}VPGEG\text{-}(VPGVG)_2]_n$ suffers strong polarity changes between its protonated and deprotonated states as a consequence of pH changes around its effective pK_a [18].

The list of external stimuli that can be used to trigger such smart behaviour is nowadays quite extensive and, in addition to thermal and pH responsiveness, includes pressure, light, redox state (electric current) and a variety of chemical potentials such as calcium or glucose concentration. A good example of this is the photo-responsive ELPs, which carry photochromic side chains either coupled to functionalized side chains in the previously formed polymer (chemically or genetically engineered) or by using non-natural amino acids that are already photochromic prior to chemical polymerization [27, 29] .

The biocompatibility of the material is a key parameter for its use in drug-delivery systems. Thus, the lack of immunogenicity already described for ELRs, along with their biodegradability and biocompatibility for human tissue, tissue fluids, and blood, make these polymers exceptional candidates as carriers in delivery systems [30-32]. The inflammatory response of ELRs has been studied by different authors using *in vivo* assays. For example, Sallach *et al.* have developed a recombinant elastin-mimetic triblock copolymer that shows a minimal inflammatory response [33]. Moreover R. Herrero-Vanrell *et al.*, also observed a poor inflammatory response when they used poly(VPAVG) as a vehicle for intraocular drug-delivery systems [34].

This manuscript will review the use of recombinamers, in particular ELRs, in the field of drug delivery which, although still incipient, has already shown its ability and ample potential. The use of recombinamers as drug depots will be reviewed in the first part of this manuscript. This includes those systems in which the drugs are either adsorbed, chemically bound or fused to them using genetic engineering techniques. The design of selected nanometric drug-delivery systems will subsequently be discussed. Finally, the paper ends with a brief look at the developments that can be envisaged for this field in the near future in order to gain a better perspective of what this approach could contribute as regards advanced drug-delivery systems. Figure 1 summarizes the

different ELR-devices for drug delivery described in the literature. Varying the composition of the ELR or ELR conjugate may result in more or less complex systems.

2. Macroscopic devices for drug delivery

Nowadays, even in cases where the patient suffers localized disease or pain (of a single organ or part of it), the treatments that are usually available to physicians involve systemic drug administration. This kind of administration is particularly suitable for acute treatments as it requires minimal expertise, although it also presents several disadvantages for long-term therapies, especially the fact that the drugs administered are distributed throughout body, including the site of action, thus meaning that a higher dosage is required to achieve the desired efficacy and consequently increasing the risk of adverse systemic side effects. Additionally, restrictions related to the molecular structure of drugs exist as very few molecules are able to overcome the physiological barriers required to reach their objectives [35]. Moreover, the rapid body clearance observed when drugs are dosed systematically (enteral or parenteral administration) means that frequent and repeated administrations are required to achieve sustainable drug delivery.

In recent years, alternative strategies have been developed to refine and improve drug delivery and therefore therapeutic efficacy. In this regard, nano- and microparticle-based delivery devices have demonstrated an ability to provide the controlled release of small molecules for periods of several weeks [36]. The local implantation of depots is one the most common methods used for drug administration, especially when long-term delivery is required. Indeed, the use of this kind of drug reservoir allows local drug concentration to be increased and sustained release to be achieved for both small and large drug molecules [36, 37]. Several drug depots have been produced in the form of a continuous solid mass or loaded hydrogels, and new approaches have been proposed to obtain less invasive implantation/removal procedures for these devices, such as the development of injectable biodegradable hydrogels that form the required depot *in situ* [38].

Hydrogels are defined as three-dimensional networks of polymeric chains that are able to swell in water but do not dissolve. The formation of 3D polymeric hydrogels was originally obtained by chemical synthesis of monomers, although this meant that no control over chain length or hydrogel structure was possible. Fortunately, recent

progress in this field has evolved towards smart devices produced using a new generation of biomaterials whose designed molecules are able to self-assemble or cross-link under physiologically friendly conditions and whose properties can be fine-tuned [39]. The properties that make this new generation of hydrogels particularly suitable for use in medical devices include their biocompatibility and soft hydrophilic structure, along with the fact that they exhibit controlled porosity, swelling behaviour, a wide range of mechanical properties, degradability, and stimuli-responsiveness to changes in their environment [40].

2.1. ELR-based depots and hydrogels

Elastin-like recombinamers are possibly the most promising candidates amongst the smart biomaterials that are currently being investigated for this purpose as these protein-based polymers can be synthesized using genetic- and protein-engineering techniques, their macromolecular structure is well defined and their sequence can be designed to calibrate their properties to better fit their required functions [41]. Moreover, most of the key properties of ELR-based hydrogels can easily be tailored to improve their performance. The porosity and swelling behaviour [42, 43] of such hydrogels is an advantageous characteristic in drug-delivery applications as it allows the hydrogel to be loaded with both small and large molecules such as DNA, growth factors or proteins that can be released at a rate dependent on the diffusion coefficient but also in response to a physiological stimulus. Indeed, their own porosity and pore size can be modulated by varying simple parameters such as concentration [44], CO_2 pressure [45] or by using gas-foaming and salt-leaching techniques [46]. Likewise, the mechanical properties of the hydrogels employed in drug-delivery applications should be adapted according to the requirement of the site where they will be implanted in the body and its mechanical stresses. Adaptation of their elastic/viscous behaviour can be achieved by adjusting the polymer concentration and crosslinking conditions [47, 48]. As described previously, all VPGXG-based ELRs present temperature responsiveness in aqueous solution depending on the chemical properties of the guest amino acid [49], properties that may also provide further responsiveness [50] to other stimuli such as pH [51], ion concentration [52] or UV-vis light [26] and that are preserved when ELR-based hydrogels are produced, thereby resulting in multi-stimuli-responsive hydrogels [53]. The tuneable stimuli-responsiveness of ELRs allows, among others, the possibility of obtaining an injectable ELR solution at room temperature that coacervates under

physiological conditions to form a local delivery depot [53]. The half-life of the resulting ELR-based coacervate at the administration site is at least 25-fold longer than for the soluble version [54]. Finally, ELRs are extremely non-inflammatory and biocompatible materials [55] and the removal of ELR-based devices when their payload is exhausted is not necessary as the biodegradation of these protein-based scaffolds follows the same natural routes as those found for structural proteins[56-58], whose degradation products, namely simple amino acids, do no present any toxicity or adverse responses[33]. Although no systematic studies on the biodegradability of ELR-based hydrogels are yet available, several parameters related to the requirements and characteristics of the body site should be taken into consideration when designing a drug-delivery device. As is the case *in vitro* [31], the *in vivo* half-life of the ELR-based hydrogel mainly depends on both enzymatic digestion and dissolution, therefore factors such as porosity, recombinamer sequence (in terms of presence of protease-sensitive sequences), and the cross-linking rate can be modified to tune their stability. For instance, the biodegradability of hydrogels obtained by coacervation may be markedly lower than that for their crosslinked counterparts as a result of dissolution. Moreover, in an environment in which ELR-based depots are exposed to a prolonged nonspecific proteolytic action, the ELRs, like all protein molecules, will be digested in a short time, whereas in less aggressive environment the half-life of such depots can exceed twelve months [33].

ELR-based depots have been developed as therapeutic agent reservoirs for use in intra-articular [31, 54] ocular [59] or infectious [60] disease, cancer [61, 62] and in diabetes [63], with some of the most recent examples being discussed below (Figure 2).

2.1.1 ELR-based depots and hydrogels for reducing systemic toxicity

Unfortunately, the most efficient therapies to deal several important diseases may affect the wellness and patient health due to their systemic side effects. The local neuroinflammation of the disc herniation, for example, can be alleviated by aggressive medications that cause moderate immunosuppression consequently, an alternative strategy of confined drug delivery is especially required. Shamji *et al*. have described tritium-labeled ELR depots that possess a local half-life eight times greater than their soluble counterparts under physiological conditions and when placed in the perineural space presented both benefits of reducing systemic free ELR drug and of preserving its

healing effectiveness [64]. The same therapeutic strategy of utilizing injectable drug gels for neuroinflammation treatment but changing the therapeutic agent was carried out to produce, depots of conjugate curcumin [65] or necrosis factor alpha inhibitor (sTNFRII) fused to an ELR carrier to invert the inflammatory response in dorsal root ganglion explants [66].

Similarly, the clinical requirement of controlling post-surgical infections by way of the local and continued release of antibiotics can benefit from the use of ELR-based depots. Bacterial infections continue to be a major complication after surgical procedures, especially in orthopaedic surgery, despite recent advances in parenteral antibiotic prophylaxis, which mostly consists in long-term intravenous antibiotic administration [67]. The delivery of antibiotics using depots can enhance the local concentration of the drug and limit systemic toxicity during wound healing. In this work, drug loading was performed by lyophilizing cross-linked hydrogels with a homogenous porous size and then hydrating them in aqueous solutions containing drugs such as cefazolin or vancomycin. The ELR hydrogels entrapped antibiotics and released them slowly and in a sustained manner (25 hours for cefazolin and approximately 1170 hours for vancomycin). The bioactivity of these devices has been assayed in an inhibition test of *Bacillus subtilis* bacterial culture and found to be similar to that of the free antibiotics during throughout the delivery time [68]. To solve the same clinical issue, Anderson *et al*. tried to improve the mechanical properties of antibiotic-collagen depots, which are unstable in aqueous solution, by mixing with an ELR in a proportion of 3:1 respectively. Non-crosslinked ELR-collagen hydrogels loaded with the antibiotic doxycycline hyclate were obtained after incubation of a solution containing all the components for 24 hours under physiological conditions [69]. The resulting ELR-collagen hydrogels showed a significantly higher elastic modulus and sustained doxycycline release over a period of 5 days. Moreover, the antibiotic released showed antibacterial activity against at least four bacterial strains of clinical interest (*Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus sanguinis*, and *Staphylococcus aureus*).

2.1.2. ELR-based depots and hydrogels for improving therapeutic efficiency

As stated above, the therapies used to date to alleviate the symptoms of chronic illness require regular and recurrent administrations, the frequency of which depends on the

ease with which the drug can reach the target tissue, the dosage required and its turn-over. The drug depot approach offers the possibility of formulation in multiple dosage forms to calibrate the delivery strategies and provide a suitable alternative for reducing the frequency of administration and, consequently, patient discomfort. Dry eye disease (DED) arises due to a chronic lack of lubrication and moisture on the surface of the eye, thereby causing pain, loss of clear vision, and increasing the risk of infection. It is one of the most common ocular diseases, affecting between 5% and 35% of the global population [70]. Continuous topical administration of artificial tear solutions, which is the common treatment for mild cases of the disease, is not appropriate for the most severe cases. An alternative and efficient therapy for this syndrome involves administration of the regulatory human tear protein lacritin, an abundant protein component of human tears that increases both lacrimal gland secretory activity and mitosis of the corneal epithelium [71]. To increase its retention on ocular surfaces in which all proteins suffer a rapid clearance, Wang *et al*. designed and produced a lacritin-ELR fusion protein, the ELR component of which was able to drive the self-assembly and phase separation of the construct and thus to form depots. The lacritin-mediated cell response in *in vitro* assays showed that this system was able to promote secretion in specific lacrimal gland cells. Moreover, *in vivo* studies performed in an NOD (non-obese diabetic) mouse model demonstrated the ability of the lacritin-ELR to stimulate tear secretion in a similar manner to the soluble drug while enhancing the local retention time in the lacrimal gland by at least six fold. The two components of the construct preserved their properties, namely the pro-secretory function of lacritin and the temperature-responsiveness of the ELR, which allowed coacervation of the fusion ELR below physiological temperature. This allowed the authors to obtain an injectable intralacrimal depot that afforded sustained delivery of the drug (Figure 3) [59].

Another ELR fusion protein has been designed to produce an injectable drug depot that can easily be injected into the knee joint, one of the tissues in which systemic administration is insufficient due to the paucity of vascularity. An increased level of cytokines in synovial fluid after joint injury is related to the onset and progression of different arthritis-type conditions [72], therefore the intra-articular administration of cytokine antagonists is a promising therapy despite the fact that their rapid turnover limits their effectiveness. With the aim of obtaining a sustained drug-eluting system, the interleukin-1 receptor antagonist (IL-1RA) has been fused with an ELR and the final construct found to conserve the desired thermal phase-transition behaviour. Although

the fused IL-1RA exhibited a reduced bioactivity than the free version, the gradual and constant depot disaggregation achieved enhanced local drug concentrations [73], thereby extending the residence time in the joint and consequently reducing cartilage degeneration at both a macroscopic and a microscopic level [74]. Recently, a different approach has been proposed to increase the persistence of cytokine antagonists without reducing functionality. Thus, Kimmerling *et al*. synthesized ELR-based cross-linked hydrogels to form a sponge-like device that was able to encapsulate both native IL-1RA and soluble tumour necrosis factor receptor II (sTNFRII) [75]. In this work, the authors compared the therapeutic effect of the continued delivery of IL-1RA and/or sTNFRII on post-traumatic arthritis remission in a murine intra-articular defect model. The prolonged release of IL-1Ra decreased post-traumatic arthritic disease by reducing the degeneration of cartilage, facilitating bone healing and decreasing synovial inflammation. Both ELR devices loaded with sTNFRII only or jointly with IL-1RA achieved the sustained delivery of therapeutic agents, although no protective effects for cartilage and bone regeneration were observed in this study due to the inhibition of tumour necrosis factor α [76].

Diabetes mellitus type II is a hyperglycaemia-related metabolic disorder that affects approximately 6% of the adult population in industrialised nations and is therefore one of the most common chronic diseases [77]. A promising alternative therapy for diabetes type II involves treatment with glucagon-like peptide 1 (GLP-1), an incretin protein hormone secreted by the gastrointestinal cells that stimulates insulin secretion and mitosis signalling pathways in pancreatic and insulinoma cells [78]. However, the rapid degradation suffered by this peptide in the bloodstream (its half-life is less than 2 minutes) limits its use in diabetes treatments.

With the aim of constructing improved, controlled-release glucose-delivery systems, Chilkoti and co-workers designed and produced two genetically fused ELRs which final products presented different GLP-1 drug cargo. The fusion constructs self-assembled to form depots and could be used in two different delivery strategies. In the first one, the ELR and the drug component are separated by a target sequence of the ubiquitous exopeptidase DPPIV which, after cleavage, releases GLP-1 monomers from the depot [63]. In order to limit the natural proteolysis of GLP-1, the endogenous DPPIV cleavage sites were eliminated by site-directed mutagenesis. The mouse model used to evaluate the *in vivo* effects confirmed that the ELR-GLP-1 construct was injectable and able to form stable depots. The GLP-1 monomers released by the animal proteases were active,

thus meaning that lower glucose levels were maintained for at least five days [63]. In the second approach, *in vivo* assays showed that a solution of GLP-1-ELR lacking the proteolytic target easily formed subcutaneous insoluble coacervates, which provided sustainable delivery of the GLP1-ELR. Sustained delivery of the GLP1-ELR was compared with both GLP1 monomer and other GLP1-ELR versions soluble under physiological conditions. Although the GLP1 released was genetically fused to the ELR, it nevertheless preserved and stabilized glucose activity and, furthermore, was able to extend the protein plasma half-life when delivered systemically. Indeed, a single injection in mice produced a five-times longer effect with respect to the soluble version and 120 times longer than that provided by the native peptide [79].

3. Nanoparticle-based devices for drug delivery

The challenge of delivering the correct concentration of a therapeutic agent at its site of action, and for sufficient time to be efficient, can be overcome by using traditional drug-loaded depots when the damage has well-localized targets. Unfortunately, many important diseases cannot be treated with a local single application that provides a prolonged and confined drug delivery. This is the case, for example, when the simultaneous treatment of different organs is required, and this is especially important in metastatic cancers. In order to reach widespread cancer cells, molecular drugs must be specifically directed and protected from natural and tumor-related barriers while in vascular circulation. To this end, over the last few years biomedicine has turned to the use of nanomaterials as smart drug-carriers, although the clinical outcome in treating these diseases is still far from optimal [80-82]. In this regard, a large number of biomaterials that are considered to be effective drug-delivery systems on the nanoscale can be found in the literature [83-85]. Several of these candidates comprise well-known polymers adapted to the new requirements although, more recently, tailor-made biopolymers with a structural design focused on each specific application are emerging as the best solution.

The ideal nanocarrier should transport the correct amount of drug to the target cell according to the criteria proposed by Lin and Cui [85]. Basically, these include sufficient drug loading, good stability during circulation to reach the target safely, selection and accumulation in the target cell and, finally, promotion of the interaction between the drug and its cellular receptor to induce the desired changes in cell status.

Although traditional biomaterials can successfully meet the first two conditions, the last ones (concerning specific interactions with the molecular components of the cell) are rarely achieved by just one type of these materials. This is due to the fact that, in both steps, nanocarriers have to recognize the natural molecules of the target cell and use them to deliver their payload correctly. These interactions have been achieved by the use of hybrid nanodevices but also with genetically engineered nanocarriers specifically designed according to the task at hand [7, 8, 82]. These innovative biopolymers offer numerous advantages in addition to those inherent to their recombinant nature (versatile structure, environmentally friendly production, monodisperse sequence, ...). In addition, they can be chemically modified and adapted as traditional materials and can interact with the cellular components in a similar manner to a natural protein. Genetically engineered carriers can be polymeric, such as polypeptides derived from elastin or silk, or non-polymeric, such as the vault and virus proteins recently reviewed by Shi *et al.* [8]. In the first case, another important characteristic turns out to be essential for their use in the synthesis of drug nanocarriers. Thus, in addition to their low immunogenicity and good biocompatibility, these polymers self-assemble, by way of a stimulus-responsive mechanism that is highly tunable in ELRs.

3.1. Nanocarriers derived from ELRs

3.1.1. ELR block copolymers

ELR-based nanoparticles were initially developed by the synthesis of tailor made di-block copolymers and the main genetic strategies used have been extensively reviewed elsewhere [4, 7, 86]. The selection of the appropriate guest residue in the amino acid sequence VPGXG and the combination of diverse blocks, with the resulting differences in their respective transition temperatures, results in ELR-based amphiphilic block copolymers. When the solution temperature is increased above the lower T_t the hydrophobic block suffers its typical transition whereas the hydrophilic block (higher T_t) remains soluble. This means that the hydrophobic moiety folds and segregates from the aqueous solution whereas the hydrophilic block remains fully hydrated in contact with the surrounding water, forming the corona of a micellar structure [87]. This means that non-polar drugs can be entrapped in the hydrophobic core, whereas the hydrophilic surface can present polar bioactive molecules [32, 88].

In addition, the number of repeats and the architecture of the ELR blocks has been shown to be useful for both tuning the T_t and selecting the type of nanoparticles [89]. This study demonstrated how the common micellar nanostructure derived from the self-assembly of ELR block copolymers can be shifted to a hollow vesicle (Dh ≈ 200nm) by changing the block lengths and arrangements. Therefore, close control over the aggregate morphology could allow the quantity and quality of the loaded drug to be varied. In contrast, more recent studies [90, 91], have reported the synthesis of cylindrical micelles or vesicles containing non-elastin assembly domains or complementary leucine zipper motifs, respectively. In the latter case, the average diameters were 1.26 and 1.82 μm while the thickness of the vesicle membrane was about 20 nm. As a proof of their suitability as drug carriers, the microvesicles were used to efficiently encapsulate both fluorescein and fluorescent polystyrene nanoparticles (diameter ≈ 500 nm), although their application *in vivo* will require a reduction in the critical salt concentration values required to form the vesicles to physiological levels [91].

Another example is the ELR diblock copolymer with triple stimulus responsiveness synthesized by Chilkoti's group [92], which responds to temperature, pH and transition metal ions in a physiologically relevant range of 32-40°C, pH 7.4-6.4 and Zn^{2+} concentration (0-25 μM). This [VG7A8]-80/[VH4]-100 diblock forms stable 60-nm micelles under physiological conditions with the histidine-rich block (VH4) at their core. pH subsequently stimulates their disassembly in the extracellular tumor pH range (≈6.8), whereas physiological Zn^{2+} concentrations stabilize them without affecting their pH sensitivity. The *in vivo* behavior of this biopolymer was examined by i.v. injection in nude mice bearing tumor xenografts. The pH-sensitive nanocarriers demonstrated a prolonged circulation half-life and around 4% of the injected dose reached the tumor after 8 h. Additionally, they presented a homogeneous intratumoral distribution and deeper penetration than pH-insensitive nanoparticles, which remain in the perivascular space because the tumor pH cannot disassemble them into the individual polymer chains. These pH-sensitive nanocarriers appear to be excellent candidates for carrying radio- and chemotherapeutical agents for which tumor penetration is a critical success factor [92].

A different strategy has been developed to increase the drug loading of the toxic and hydrophobic immunosuppressant rapamycin (Rapa) into nanoparticles [93, 94]. In these

studies, a serine-based ELR block was flanked by an isoleucine-based block and the FK506 binding protein (12KDa), the human receptor for Rapa. Due to its low water solubility and use of the two-phase solvent encapsulation method, the drug was entrapped in the core of the nanoparticle in addition to being retained in the corona. This dual strategy increase the drug loading to 75%, the solubility at least 10-fold and the *in vitro* half-life release from 2 h to nearly 60 h. The Rapa-ELR nanoparticles were successfully tested in two mouse models. Thus, i.v. injection in a human breast cancer model produced a drastic reduction in tumor growth and lower toxicity than free Rapa [94]. Similarly, their use in a mouse model of Sjögren's syndrome suppressed lymphocytic infiltration in the lacrimal gland and reduced cathepsin S levels [93].

In a recent paper, the same research group used a previously described chimeric ELR [95] to target a completely different tissue [96]. In this case the ELR diblock copolymer contained a fiber capsid protein of adenovirus serotype 5 directed to the coxsackievirus and adenovirus receptor (CAR), a cell adhesion protein present in liver and lacrimal gland cells. The *in vitro* internalization of ELR nanoparticles was successfully demonstrated in liver cells in the former study, whereas in the latter they were internalized and transported from basolateral to apical membranes of the lacrimal gland acinar cells after intra-lacrimal injection in a mouse model. This transcytosing property could be used in future for sustained delivery of drugs to the ocular surface by i.v. or s.c. nanoparticle application.

3.1.2. ELR monomers

The synthesis of fusion proteins comprising ELRs in *E. coli* can include therapeutic agents when they are polypeptides with simple structures (e.g. toxic peptides or monomeric growth factors); otherwise, physical encapsulation is the preferred option [97-100]. When this is the case two aspects become critical: the polypeptide must conserve its biological function in the fusion protein and the ELR block must retain its ability to self-assemble. The specific fusion protein comprising monomeric keratinocyte growth factor (KGF – Mw 22.5 kDa) and a hydrophobic ELR (T_t 28°C) produced stable nanoparticles (Dh 530nm) under physiological conditions [101]. When the activity of the recombinant growth factor was assessed, the *in vitro* cellular test showed a similar keratinocyte proliferation rate in the presence of fusion protein as for free KGF. In

contrast, the molecular activity (measured as substrate phosphorylation) was clearly reduced when the KGF was fused, thus indicating that its aggregation hinders interaction with the receptor without compromising the overall cellular effect. The *in vivo* healing results in genetically diabetic mice showed that the KGF-ELR nanoparticle enhanced granulation tissue and improved re-epithelialization when compared with free factor. An alternative to the fusion proteins with monomeric growth factors using the single-chain vascular endothelial growth factor was described more recently [102]. In this work, both molecules were non-covalently linked by a coiled-coil association and the nanoparticle was found to be effective against cancer cells by inducing cell apoptosis.

The advantages of fusing therapeutics to ELR-based carriers are numerous. Thus, these polypeptides can be easily expressed and purified from *E. coli* sources, which makes it possible to produce huge amounts of the fused peptide while avoiding any putative toxicity [103-110]. Additionally, as mentioned above, the intrinsic self-aggregation property of ELRs can be exploited for several purposes [32, 111, 112]. Finally, yet perhaps more importantly, ELR polypeptides are macromolecules with a long plasma half-life [94, 113], which stabilizes circulation of the fused peptide, and a remarkable *in vivo* biocompatibility [33, 114] and biodegradability [115] that allow their use as long-term depots with a minimal inflammatory response.

Recently, a combined strategy has demonstrated promising results against pancreatic cancer [108]. The authors fused two therapeutic peptides to the ELR for a local therapy by mild hyperthermia, and combined it with a systemic treatment with Gemcitabine, a pyrimidine nucleoside analogue of cytarabine. The ELR fragment was flanked by a *Bac* cell-penetrating peptide, which mediates intracellular uptake of the nanoparticles, and by a cell cycle inhibitory p21-derived peptide that leads to arrest at the G1 and G2 or S phase of the cancer cell cycle. The chimeric polypeptide efficiently inhibited the growth of three pancreatic cancer cell lines and its activity was enhanced by combination with gemcitabine. However, this synergism in inhibiting tumor growth with the combination treatment was not achieved in the xenograft mouse model, probably because additional studies to adjust the dose ratios between two drugs are required.

The inclusion of bioactive peptides has also been used for the synthesis of ELR-based nanoparticles for DNA encapsulation. Plasmid condensation was initially accomplished by the fusion of a cationic oligolysine peptide with the ELR [116]. When the block

copolymer was incubated with a plasmid containing the Green Fluorescent Protein gene, the resulting polyplexes (Rh ≈ 100 nm) were able to transfect cells and its cytotoxicity was clearly reduced. Therefore, the ELR moiety induced formation of the nanoparticles as well as neutralization of the intrinsic cytotoxicity of the oligolysine peptides. In contrast, in a recent paper, a plasmid-condensing peptide (RH3) was fused to four different and short ELRs, varying their guest residue [93]. All biopolymers were able to condense pDNA into nanoparticles (Rh <200 nm) and their immunogenicity was analysed by repetitive injections into immunocompetent mice. The IgG response showed that, in contrast to those containing K and E, the less immunogenic ELRs were those including non-charged guest amino acids (S and A). Finally, functional peptides (penetratin and LAEL fusogenic peptides) were fused to a positively charged ELR and their cellular transfection ability tested [117]. The resulting biopolymers condensed the plasmid DNA and the polyplexes (Dh 150-300nm) were internalized by cancer cells via endocytic pathways and were able to transfect them, especially when both fusogenic peptides were present in the same molecule (Figure 4). Interestingly, all the ELR polyplexes showed better biocompatibility when compared with the cationic polymer PEI. These results demonstrate the utility of ELRs as biocompatible non-viral systems for gene-therapy applications.

Recently, alternative approaches to tuning the polymer transition, and thus nanoparticle assembly, have been studied [118]. One successful strategy for overcoming this limitation is the chemical conjugation of therapeutic non-protein molecules (doxorubicin, paclitaxel, salinomycin, etc.) to the ELRs [119-121]. In these examples, the hydrophobic drug triggers assembly of the chimeric molecules into micelles and the ELR polymer protects the therapeutic load until it reaches its cellular targets. The roadmap described by Chilkoti should be consulted for a rational design of thermally responsive drug-loaded nanocarriers [122]. This work provides a simple predictive model for selecting drugs suitable for inducing conjugation-triggered self-assembly and for entrapment in the nanoparticle core. Recently, a new model has been described in order to design drug-loaded ELR nanocarriers for an alternative targeting strategy: so-called “heat seeking” nanoparticles, which thermally target an externally heated tumor [123]. As the approved temperatures for mild hyperthermia of solid tumors range from 40 to 45ºC, this work presents a mathematical model for predicting the T_t of the drug-loaded nanoparticles and selecting those with T_t’s between 39 and 42ºC *a priori*. This

model has been successfully tested in a colon carcinoma mouse model with Doxorubicin-conjugated ELRs.

A combined encapsulation strategy was assessed by Zhao *et al*. with the aim of increasing drug loading in ELR-based nanocarriers [120] Thus, salinomycin was chemically conjugated to cysteine residues and free drug was also physically entrapped. The covalently attached drug induced the synthesis of stable nanoparticles surrounded by ELRs, whereas the entrapped drug increased the drug loading up to 25%. Additionally, two additives were added to increase the load up to 75%, with the first neutralizing the negative charge present in the drug and the second increasing the hydrophobicity of the nanoparticle core. This complex nanocarrier was found to be cytotoxic in a cancer cell line, with an extended *in vitro* drug delivery half-life of 4 h, a 5.2-times longer *in vivo* plasma half-life and higher accumulation in the tumor. However, this nanodevice did not inhibit tumor growth more effectively than free salinomycin, probably due to the intrinsic characteristics of the breast tumor selected.

The performance of ELR-derived nanocarriers in cancer treatment has recently been compared with a nanoformulation of the highly hydrophobic drug placitaxel (PTX) approved by the Food and Drug Administration [121]. This nanomedicine (Abraxane) consists in a 130-nm diameter particle of PTX physically bound to human serum albumin. In contrast, PTX was chemically conjugated in the ELR-PTX nanoparticle via a pH-sensitive linker and the Rh in PBS was around 32 nm. Both nanoparticles were tested in terms of their *in vitro* and *in vivo* anticancer efficacy and compared with free PTX in human prostate and triple-negative breast cancer models. The *in vivo* plasma exposure of ELR-PTX nanoparticles was seven-fold higher than free drug and two-fold higher than Abraxane, thus meaning that the ELR-PTX concentration in the tumor was 2.5-fold higher than for free PTX, whereas ELR-PTX accumulation in healthy organs was clearly lower than for Abraxane, particularly in muscle and liver. When the different formulations were compared in the cancer models, a single intravenous injection of ELR-PTX nanoparticles showed better tumor regression than Abraxane in the breast model and, while the Abraxane-treated mice bearing the prostate tumor survived ≤60 days, 100% of the mice injected with ELR-PTX survived for >70 days. These results, along with the inherent features of the genetically engineered nanocarriers, provide new reasons for testing their prompt translation into clinical practice [121].

3.2. Nanocarriers derived from SELRs

Silk-like proteins (SLPs) are another type of recombinant material with demonstrated success in biomedical applications [124, 125]. These biopolymers are designed taking into account the repetitive peptide sequences found in silkworm and spider silk. The most common of all silk variants is probably the hexapeptide GAGAGS from *Bombyx mori* fibroin, although recombinant spider silk has also been used in nanoparticles for gene and drug delivery [126, 127]. In aqueous solution, these silk-derived polymers undergo an essentially irreversible conformation transition from random coil to beta sheet and a subsequent beta sheet aggregation growth accelerated by an increase in temperature. The good biocompatibility and biodegradability shown by these materials, and particularly the mechanical strength of the resulting aggregation products, stimulated the design of chimeric materials such as silk-elastin like recombinamers (SELR). There are many examples of the use of SELRs in drug delivery and these have been extensively reviewed in the last few years [9, 128, 129]. However, very few studies reporting the synthesis of SELR-derived nanoparticles have been published [130-133]. These studies demonstrated that the self-assembly of SELR into spherical nanoparticles is a process in which the length of the silk block determines both the kinetics and the size of the aggregates. Thus, an initial temperature-driven aggregation mediated by the elastin block forms nanoparticles (around 40 nm in diameter) that then self-assemble into a nanofibrillar morphology in an annealing time-dependent manner (Figure 5). This coordinated and concomitant dual-gelation mechanism leads to the final maturation into a resistant hydrogel made of the fibrous structures when the concentration of the material and annealing time are appropriate [131, 133]. Consequently, the use of SELRs in nanocarrier synthesis for drug delivery has excellent potential but requires more in-depth studies of the production procedures and choice of the final target.

The first attempt to investigate the applicability of self-assembled SELR nanoparticles in drug delivery was described recently by Xia *et al.* [132] with the antitumor drug doxorubicin in an *in vitro* system. In this work, three previously described SELR constructs [131], with different ratios of silk component, were incubated with DOX, which triggered their self-assembly into micellar-like nanoparticles (with Rh ranging from 50 to 140 nm) at 25ºC (the T_t of the elastin component is >27ºC). The drug-

loading efficiency was higher for the shorter silk blocks in the SELR and the nanoparticles were found to be stable for 48 h under physiological conditions, with a minor increase in size and polydispersity. As regards *in vitro* studies with the HeLa cancer cell line, the SELRs with no drug were found to be non-cytotoxic up to 0.2mg/ml whereas the IC50 for drug loaded-nanoparticles was lower the shorter the silk block, with a 1.8-fold higher cytotoxicity than the free drug. This indicates that DOX internalization was modulated by the SELR and the importance of the selection of the silk-block for appropriate drug delivery [132].

3.3. Hybrid nanocarriers containing ELRs

The versatile and exclusive characteristics of ELRs, along with their chemical properties, have catalyzed the emergence of hybrid compositions with traditional materials. These new BioHybrid materials are synthesized by conjugation of organic or inorganic substances with the appropriate ELRs and present promising opportunities in the drug-delivery field. Relevant examples of this innovative strategy involving polysaccharides, lipids, silica and metals can be found in the literature from the last few years. Thus, multicompartmental capsules were formed using the layer-by-layer method by combining polysaccharides (chitosan and alginate) and an RGD-containing ELR that confers both stimuli-responsiveness and cell adherence on them. Their application as drug-delivery devices was demonstrated when the release profile of the entrapped rhodamine was found to be temperature dependent [134].

ELRs containing thermosensitive liposomes (e-TSL) are composed of four different components, namely a phospholipid, cholesterol, PEG and ELR-lipid conjugates, and their potential use was studied using calcein and doxorubicin [135]. The optimized liposome formulation (Dh 161 nm) encapsulated DOX and was tested using mild hyperthermia as a stimulus for drug release. The blood half-life of DOX increased in mice from 12 min (free DOX) to 2 h when entrapped in the e-TSL and showed superior drug accumulation in tumors. Finally, the e-TSL exhibited anticancer properties in a murine mammary tumor model with a hyperthermia-dependent effect.

Two hybrid organic-inorganic materials based on thermosensitive ELRs have been synthesized recently. Thus, silica micelles are formed when an ELR diblock copolymer is expressed containing a biomimetic silaffin peptide on their coronae. Subsequent

additional condensation of the silica resulted in highly negative monodisperse nanoparticles (Rh ≈ 35nm) that appear to be promising for encapsulation of therapeutic or imaging moieties [136]. Similarly, the chemical conjugation of an ELR on the periphery of a dendrimer produced thermally sensitive nanoparticles that were loaded with photothermogenic AuNPs. These photothermal dendrimers bound to cells and induced photocytotoxicity in a temperature-dependent manner [137].

4. Drug-ELR conjugates for drug delivery

A large number of studies have attempted to improve the bioavailability and pharmacokinetics of small-molecule drugs by conjugation thereof to ELRs, with a reduction in the therapeutic dose and an increased drug efficiency being the expected benefits of such an approach. ELRs show certain intrinsic advantages for this purpose, one of which is their biodegradability as they degrade into natural amino acids, thus allowing the use of high MW ELRs, even above the renal clearance limit, without the risk of accumulation.

Other interesting properties of ELRs that are often exploited to increase their delivery efficiency are their temperature sensitivity and ability to self-assemble. The latter is particularly relevant as regards spontaneous assembly into nanocarriers. Fortunately, several studies have shown that conjugation of the drug is not detrimental to these beneficial properties of ELRs. Indeed, in some cases, they can even be improved and tuned as a consequence of drug conjugation. One example of this is hyperthermia therapy for cancer, where it is already been demonstrated that a precise heating of deep-seated tissues can be used to thermally target ELRs to internal organs by taking advantage of the thermal sensitivity of ELRs.

The use of recombinant DNA techniques to produce these ELRs allows the easy incorporation of peptide-based functional domains that greatly contribute to the final goals of increasing the amount of drug delivered to the desired tissue or affected area, while decreasing the drug exposure experienced by healthy tissues. In this sense, although the molecular composition of ELRs tends to be quite complex, in contrast to other chemically synthesized macromolecules, the complexity in these compounds is not strongly related to increasing production time and cost [138].

4.1. Drug-ELR conjugates as chemotherapeutic agents

Different drugs have been conjugated to ELRs, with doxorubicin (Dox), a well-known chemotherapeutic anticancer agent, being one of the most widely used. Indeed, the conjugation of Dox to ELRs via acid-labile hydrazine bonds has been proven to be highly efficient as, once the nanocarriers used as drug-delivery systems have been internalized by the cells, the resulting systems are able to release their drug load in the acidic environment of lysosomes, with negligible amounts of Dox being released into the external medium of the cell [139]. In this initial work, the conjugation of Dox to the thiol group of a single cysteine in the ELR was carried out via different pH-sensitive, maleimide-activated hydrazone linkers. The linker structure and length had little effect on the T_t of the resulting ELR–Dox conjugates, all of which exhibited similar T_t's to that of the native ELR. However, ELR –Dox conjugates with longer linkers exhibited slower transition kinetics compared to those with shorter linkers. The highest release achieved upon cleavage of the hydrazone bond of the ELR –Dox conjugate at pH 4 was nearly 80% over 72 h and was provided by the conjugate with the shortest linker.

In a different study, an ELR-Dox system linked, as above, via an acid-labile hydrazone was shown to be endocytosed by squamous cell carcinoma cells (FaDu) and trafficked into lysosomes. Both the ELR–Dox conjugate and the free drug exhibited almost identical *in vitro* cytotoxicity, although their subcellular localization was differed significantly. Thus, the free drug was largely concentrated in the nucleus, whereas the conjugate was dispersed throughout the cytoplasm with limited nuclear accumulation. These differences suggest a different mechanism of cytotoxicity for the conjugate as compared with the free drug [140].

The ability of cancer cells to become simultaneously resistant to different drugs, a trait known as multidrug resistance, remains a major obstacle for successful anticancer therapy. One major mechanism of resistance involves cellular drug efflux by expression of P-glycoprotein (P-gp). However, P-gp mediated resistance can often be overcome by using P-gp inhibitors, synthesising novel analogs, or conjugating drugs to macromolecular carriers in order to circumvent the efflux mechanism. Along these lines, studies have been carried out to compare the cytotoxicity of different

macromolecular ELR-derived therapeutic agents (Tat- ELR -GFLG-Dox). These studies showed that ELR-bound Dox was equally cytotoxic in both sensitive and resistant cell lines (MES-SA/Dx5). Indeed, in contrast to free Dos, which was rapidly pumped out by the P-gp transporter, ELR-bound Dox was shown to accumulate in MES-SA/Dx5 cells. In these conjugates, the ELR was flanked with a Tat cell penetrating peptide at the N-terminus and a GFLGC cathepsin cleavage sequence by cassette mutagenesis [141]. The cell internalization mechanism observed for the ELR was shown to be mainly endocytotic in nature [142]. This particular study tested two different versions of the conjugates (Tat- ELR -GFLG-Dox), one containing ELR1, which showed thermal responsiveness at near physiological temperatures (T_t=40°C), and one containing ELR2, which does not aggregate at the hyperthermia temperature (T_t=65°C). Focused hyperthermia above a specific transition temperature at the tumour site caused the ELR to aggregate and accumulate, thereby increasing the local concentration of the drug load. The ability of Tat- ELR-GFLG-Dox to be thermally targeted in the resistant MES-SA/Dx5 cell line was also confirmed. Application of hyperthermia to Tat- ELR1-GFLG-Dox increased the drug's toxicity (nearly three-fold) and apoptosis, whereas no toxicity increase was seen with the non-thermally sensitive control construct Tat-ELR2-GFLG-Dox, thus indicating that the effect observed was due to aggregation of the polypeptide. The toxicity enhancement was 20-fold higher in the sensitive cell line.

Paclitaxel (Ptx) has also been used in conjugation with an ELR. As above, Ptx shows poor aqueous solubility, and was therefore grafted to the ELR to obtain an acid-sensitive paclitaxel prodrug for the potential treatment of breast cancer [143]. This work showed that free Ptx is more effective than the macromolecular conjugate SynB1-ELR1-Ptx in inhibiting the proliferation of sensitive MCF-7 cells. However, for resistant MCF-7_{Tax} cells, although the difference decreases substantially, the toxicity of free Ptx is much higher, thereby suggesting the existence of alternative resistance factors to the P-gp pump that cannot be overcome by polymer-based delivery. Finally, under hyperthermia conditions, SynB1- ELR1-Ptx induced apoptosis in a manner similar to conventional paclitaxel in MCF-7_{TAX} cells. This finding can be attributed to an increase in the intracellular concentration of SynB1- ELR1-Ptx. It is well known that the use of SynB1 as the CPP promotes internalization via adsorptive-mediated endocytosis [144].

The demonstration of the efficacy of ELR-Dox conjugates *in vivo* was a remarkable breakthrough in this field. One of the first preclinical studies to demonstrate the efficacy

of the thermal targeting of an ELR-Dox compound was reported by Shama Moktan *et al*. [145]. Thus, the thermal targeting of SynB1- ELR1-Dox (consisting of a cell-penetrating peptide at the N-terminus and the 6-maleimidocaproyl hydrazone derivative of Dox at the C-terminus of the ELR), in combination with induced hyperthermia, resulted in complete inhibition of tumour growth and a substantially higher therapeutic benefit of the drug in an animal model (E0771 syngeneic mouse breast cancer model). This cancer model is otherwise only partially responsive to standard Dox treatment. The advantage of combining hyperthermia with ELR *in vivo* is that hyperthermia increases the permeability of tumour vasculature compared with normal vasculature, thereby resulting in enhanced extravasation of macromolecules. Another remarkable conclusion of this work was that Dox was found in the heart of animals treated with free doxorubicin, whereas no detectable levels of Dox were found in animals treated with ELR-Dox, thereby indicating a good correlation between tumour targeting and a reduction in the potential cardiac toxicity of ELR-Dox.

The final example in this section is the conjugation of radioactive Iodine (^{131}I) to different ELRs to use such conjugates as radiotherapeutic agents. Thermal responsive ELRs have been conjugated to such isotope to create injectable depots. That allowed the location, by injection, of the radionuclei in the bulk of advanced-stage cancers which reduced the bulk of those inoperable tumours, enabling surgical removal of de-bulked tumours [61, 62].

4.2. Drug-ELR conjugates with self-assembling capabilities upon conjugation

The packaging of drugs into nanoscale delivery vehicles (diameter of 10-100 nm) is of particular interest for cancer therapy, especially as numerous studies have shown that objects within this size range accumulate within solid tumours due to the enhanced permeability thereof and the retention effect, which results from abnormalities in tumour blood and lymphatic vasculature. In this sense, different studies have focussed on the influence of the size and heterogeneity of ELR-Drug nanocarriers on their therapeutic efficiency. In the case of ELRs, the formation of such nanocarriers relies exclusively on their temperature-triggered self-assembly, thus meaning that handling and fabrication of the delivery system is greatly facilitated.

Numerous attempts to discover the rules that drive self-assembly in ELR-Drug conjugates, with the aim of using this information to rationally design nanoparticles that

also exhibit thermal responsiveness in the clinically relevant temperature range of 37–42°C and under physiologically relevant conditions, have been made over the last few years [122]. To set the basis for this dependence, fourteen different maleimide derivatives of small molecules, with a broad range of hydrophobicities, were covalently attached to an ELR identified as CP and comprising a hydrophilic ELR segment and a short Cys-rich segment that provides thiol groups for conjugation with the maleimide derivatives. The findings of this study clearly demonstrate that the attachment-triggered self-assembly of CP was possible for a wide range of hydrophobic small molecules. They also revealed a simple predictive rule that governs the self-assembly of CPs and is based on a threshold hydrophobicity of the conjugated small molecule. Thus, although the LogD>1.5 threshold predicts whether self-assembly will occur, the size and shape of the resulting nanoparticles seems to depend on the molecule's molecular structure rather than its hydrophobicity. The validity of this model was confirmed using three therapeutic drugs, namely gemcitabine (LogD=2.2), oxycodone (LogD=1.2), and Ptx (LogD=4.0), which were conjugated to the CP. Only Ptx conjugates were able to form nanoparticles, which had a similar size to those formed when using CP alone (prior conjugation). A significant increase in the solubility of Ptx was also found and, in addition, this work also showed that a rational tuning of the aggregation temperature was possible, which is also remarkable as the ability to design an aggregation temperature of between 38 and 42°C should result in a conjugated nanoparticle-drug delivery system that can be targeted to diseased tissue by externally applied, focused mild hyperthermia.

MacKay *et al*. [146] studied the efficiency of previously reported CP-Dox conjugates that spontaneously self-assemble into sub-100nm-sized, near-monodisperse nanoparticles as chemotherapeutic systems in a murine cancer model. This study proved that an efficient release of the drug takes place at pH 5 (68±3%), with an essentially insignificant release at pH 7. These findings confirm that the Dox covalent bond is exceptionally stable at physiological pH, but that this bond cleaves at an appreciable rate at a pH that is relevant to endolysosomal trafficking. CP-Dox nanoparticles have a fourfold higher maximum tolerated dose than free drug, and, remarkably, induce nearly complete tumour regression after a single dose. The median survival time for mice treated with PBS was 21 days, and treatment with Dox slightly increased this survival to 27 days. In contrast, the CP-Dox nanoparticles cured eight of nine mice for up to 66

days after tumour implantation. Thus, CP-Dox nanoparticles provide a substantial curative effect in that model.

The former systems have subsequently been further improved as the recombinant production of the macromolecular component–the ELR–paves the way to a substantial number of different modifications. One of these was the addition of tumour targeting capabilities by the inclusion of tumour-homing ligands [147]. In this work the tumour homing ligand F3 specifically binds to the nucleolin expressed on the membrane of the cells and tumour cells [67]. After binding to nucleolin, the complex internalizes into the targeted cell. Additional translocation properties have been included by incorporating translocating peptides into the ELR design. The resulting ELR (F3-ELR-C8) was finally conjugated with Dox, to give F3-ELR-C8-Dox, via an acid-labile hydrazine linker (Figure 6.a). The self-assembly of this conjugate gives rise to nanoparticles that present the hydrophilic tumour-homing peptide on the outer surface of the micelle, thereby facilitating its targeting capacity. The drug is located in the hydrophobic core with an efficient increase in solubility (Figure 6.b).

The behaviour of these F3- ELR-C8-DOX nanoparticles, which have a mean hydrodynamic radius, Rh, of 28.1±0.7nm, suggests that the pH-dependent release of DOX from the conjugates takes place by endolysosomal release of the drug after cellular uptake of the nanoparticles. More importantly, these nanoparticles showed significantly improved pharmacokinetics, optimized biodistribution, low systemic toxicity and excellent *in vivo* anticancer efficacy in a murine cancer model. Thus, they exhibited a 4.2- and 1.8-fold increase in drug levels in tumours compared with free DOX and ELR-C8-DOX, respectively, which translates into significantly enhanced anti-tumour efficacy. Similarly, they exhibited a 2.0- and 1.1-fold decrease in drug levels in the heart with respect to free DOX and ELR-C8-DOX, respectively, which translates into significantly reduced cardiotoxicity.

5. Conclusions

As discussed in the previous sections, the future perspectives for the use of ELRs and macromolecular peptide systems mostly based on ELRs are remarkable. Thus,

recombinant materials, especially those based on ELRs, show a substantial set of properties that are rarely, if ever, found together in any other class of materials currently used, or even explored, for drug-delivery applications. The clear evolution and maturation in the field of ELRs for drug delivery observed in recent years has allowed numerous decisive steps to be taken in the right direction. Consequently, the establishment of strong fundamentals in this field, as described above, will provide the basis for future developments. Similarly, the exploration and exploitation of the exceptional opportunities provided by the use of recombinant techniques has allowed the creation of complex materials and drug-delivery systems that break though current barriers and has opened up the way to new and more effective therapeutic agents. A nice example of this potential is the FSI recombinamers described by Shah *et al*. [93]. FSI is a fusion recombinamer comprising an amphiphilic diblock ELR and the rampamycin binding peptide FKBP12. The ELR part causes the recombinamer to self-assemble into nanometric micelles, whereas the FKBP12 domain increases the rapamycin binding capacity and controls its release. The system has shown potent therapeutic activity in suppressing autoimmune dacryoadenitis in a mouse model of Sjögren's syndrome. Another interesting example is provided by Wang *et al*. [148]. The LSI recombinamer described in that work, namely an ELR fused with lacritin, showed an extraordinary capacity for wound healing of the corneal surface by combining the thermal self-assembly of the ELR with the selectivity provided by lacritin. Even more surprising is the example given by Hsueh *et al*. [96]. In that case, the ELR was fused to the adenovirus type 5 fibre knob peptide. This approach opens up the possibility of a tear-mediated delivery of drug-loaded nanoparticles by transcytosis of the lacrimal gland, which is a novel and promising strategy to drug targeting in tears that overcomes the barriers to ocular delivery. Indeed, there are many other interesting examples, such as the ELRs used as drug carriers for the ocular [148], infectious [68], cardiovascular[149], joint [74] and metabolic [79] diseases described above or maternal delivery and the prevention of foetal exposure [112], thus indicating that the range of ideas and possibilities open in this field is booming and is likely to lead to the future development of a core of novel strategies that will surely transform our current view of drug delivery.

Other fronts in which ELRs are showing an extraordinary momentum include anticancer drug delivery, with many different novel strategies, used alone or in combination, including tumour homing and intracellular accumulation, as described above. In

addition, relevant studies in the field of therapeutic gene delivery have appeared recently. Novel bespoke ELRs have proven their value from simple approaches to decreasing the immune visibility of more conventional polyplexes [114] to being the vector systems themselves. An example of this is the study by Piña *et al*. [117] or that by Price *et al*. [150] in which sophisticated ELRs and SELRs (silk-elastin-like recombinamers) are described, respectively. More evidently, the work by Dash *et al*. shows how far the state of the art has progressed as regards the therapeutic delivery of genes by ELR-based systems. This work describes a nice dual system in which two different therapeutic plasmids (eNOS and IL-10) are delivered in a temporally controlled manner [151].

Therefore, in conclusion, the applications of ELRs in the field of drug delivery are rapidly evolving. Their success relies on three basic pillars. First, the easy and affordable creation of complexity at the molecular level provided by the recombinant approach. Second, the ease with which biological function, provided by functional peptides, is incorporated into the molecular designs and, finally, an improvement in our fundamental understanding of the relationship between molecular composition and biological and physical function. These three pillars are enabling a rapid development in this field and confer a clear competitive advantage on these materials with respect to other families of materials. The most important drawback hampering the achievement of the full potential of these novel materials is the limited number of groups with expertise in the recombinant production of materials, which is much fewer than those devoted to more conventional ways of materials synthesis. However, this number is clearly increasing as many researchers either spin-off from their original groups to establish new groups or other researchers coming from different areas of expertise are attracted by the potential of recombinant materials and make the transition. As such, and despite the highly promising results that can already be found in the literature, the use of recombinamers in drug-delivery systems and other biomedical fields remains in its early infancy. The impact of a more mature field will undoubtedly be huge and the next few decades will surely bring recombinamer-based drug-delivery systems that we cannot currently even imagine, with the recombinamers acting as a key player in this and other biomedical fields.

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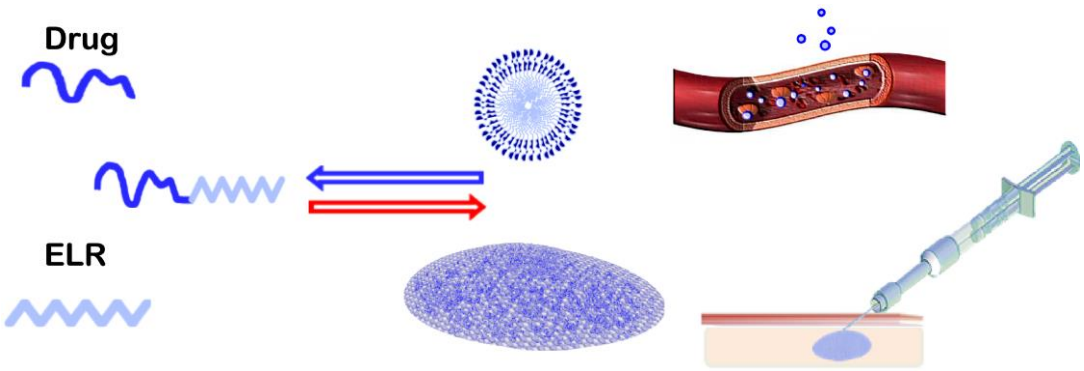

Graphical abstract

Figure 1. Schematic overview of the different drug delivery ELR-devices

A: ELR-based cross-linked hydrogels. Scheme of the strategies of using sponge-like ELR devices able to load high drug quantities. Cross-linkable ELRs molecules forms tunable hydrogels that can be loaded with the therapeutic agent (represented as purple oval). After the local implantation of the device, a long-term delivery is achieved.

B: Scheme of the ELR-based depots formation. By genetic-engineering, the therapeutic peptide (represented as pink wavy line) is fused to ELRs (represented as blue wavy line) separated by a protease-sensitive motif (represented as double blue triangle). The fusion molecule, under physiological conditions, spontaneously coacervates forming a depot that causes a local and continued release of the therapeutic agent.

C: Process of intracellular drug release from a complex ELR-conjugate containing the drug, (pink oval), a cell penetrating peptide (green arrow tip) and an acid-labile linker (double blue triangle).

D: ELR block copolymers with hydrophobic drugs. The ELR diblock self-assembles over the specific temperature transition of the hydrophobic block (red line) and then entraps the drugs increasing their solubility and bioavailability. The hydrophilic ELR block (blue line) remains unfolded and surrounds the nanoparticle preventing its aggregation. The resulting nanoparticle can transport the drug and its release may be performed by a simple diffusion mechanism. The hydrophilic block can also include targeting peptides in order to direct the nanoparticle to the correct organ or cell.

E: ELR for gene delivery. The nucleic acids (e.g. a plasmid in the figure) can be complexed by electrostatic interactions with a cationic ELR. The resulting polyplex

presents a net positive charge in the corona preventing its aggregation and inducing its interaction with the negatively charged plasma membrane. The ELR can include targeting or endosome escape peptides (green arrow head) to facilitate the internalization of the nanoparticle and the transport of the intact nucleic acid into the nucleus.

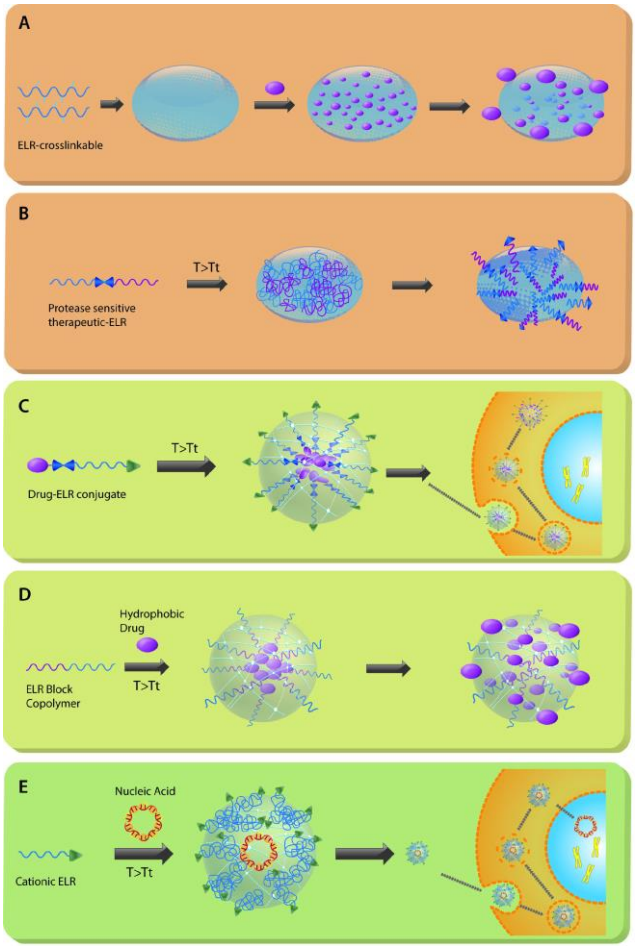

Figure 1: Schematic representation

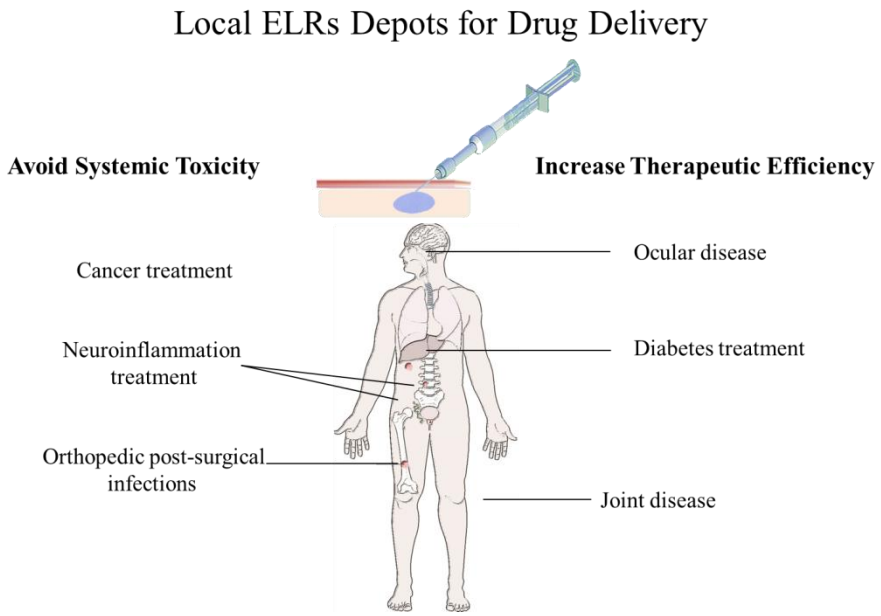

Figure 2. Scheme of the proposed drug-ELR-based depots used for therapeutic purposes. The injectable ELR-drug solution at room temperature coacervates under physiological conditions to form a local delivery depot. This strategy presents the advantage of decreasing adverse systemic side effects of aggressive therapies and/or improving the effectiveness by both reduce the drug turnover as increase the local drug concentration.

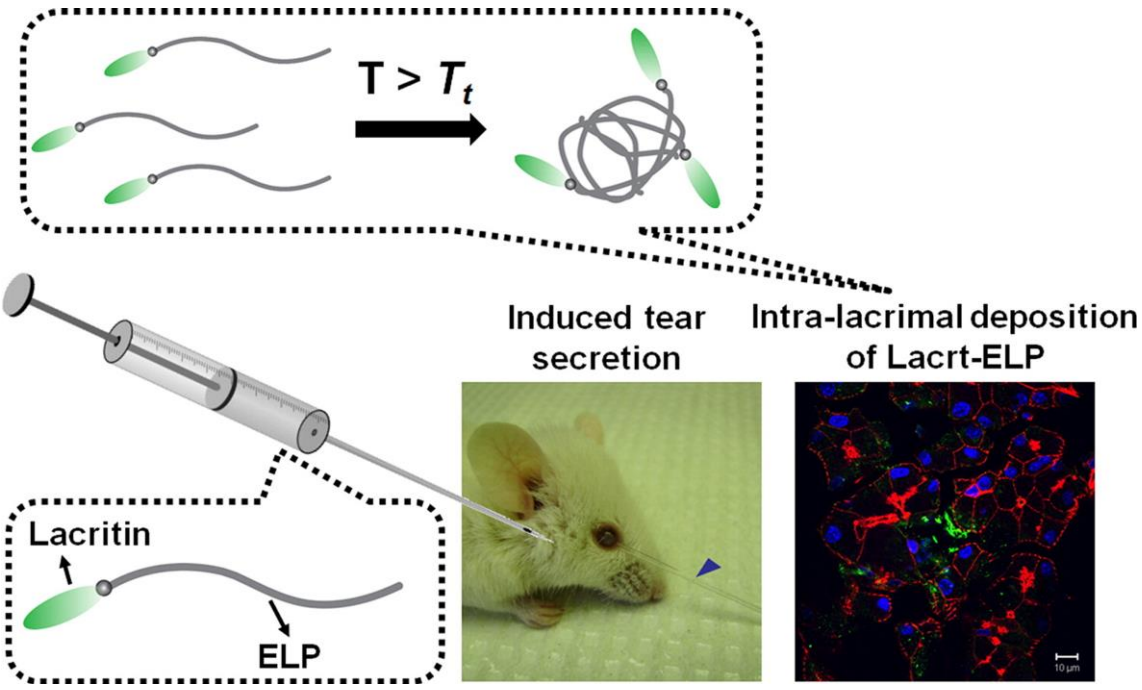

Figure 3: Schematic representation of the injectable lacritin-ELR depots formation in the lacrimal gland[59]. Reproduced with permission from Elsevier Publications.

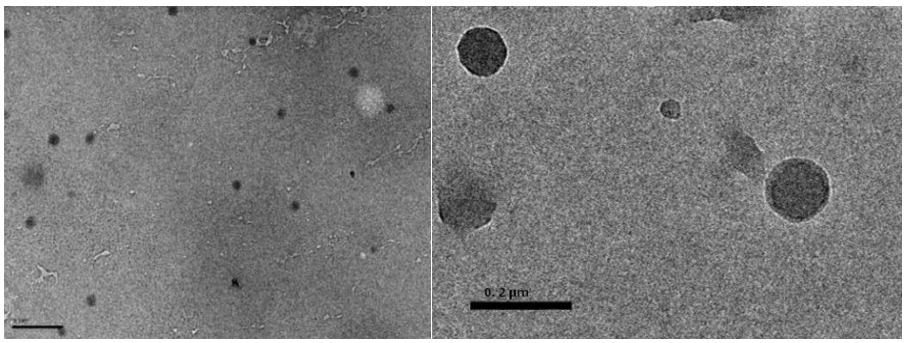

Figure 4. TEM Images for polyplexes formed by the cationic ELRs: CPP-ELR (left side) and LAEL-CPP-ELR (right side), and a plasmidic DNA. Bar scale represents 200 nm. Reproduced with permission from Wiley Periodicals, Inc[117].

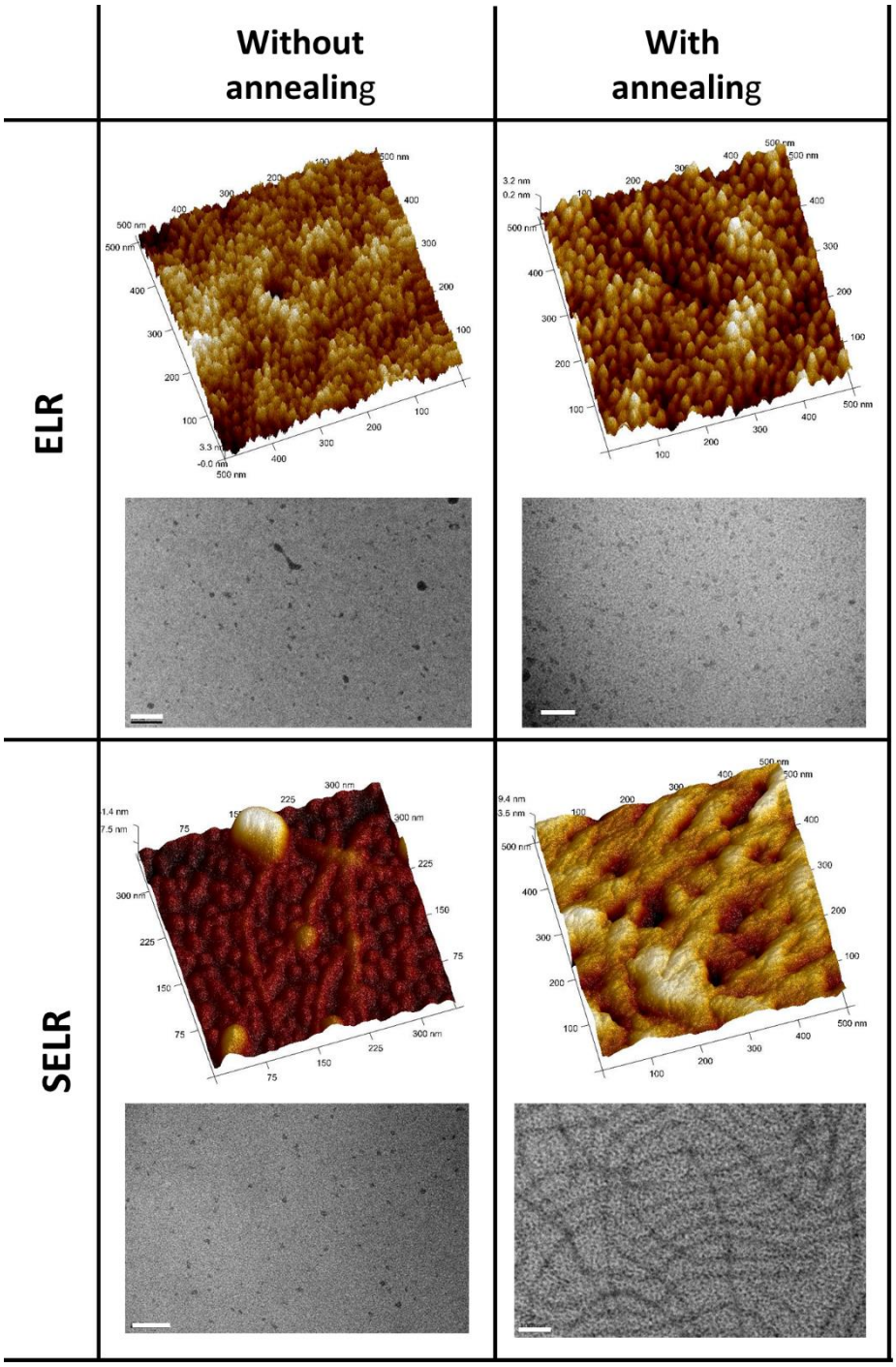

Figure 5. TEM and AFM images of the self-assembled nanoparticles formed by (EI)x2 ELR (up) and (EIS)x2 SELR (down) after different annealing conditions. Images show that SELR evolves from nanoparticles to a fiber-like state whereas the ELR displays a micellar structure in both conditions. Scanning windows are 0.5 x 0.5 μm in AFM images and the scale bar of TEM photographies represents 100 nm. Adapted from reference[133]. Reproduced with permission from ACS Publications.

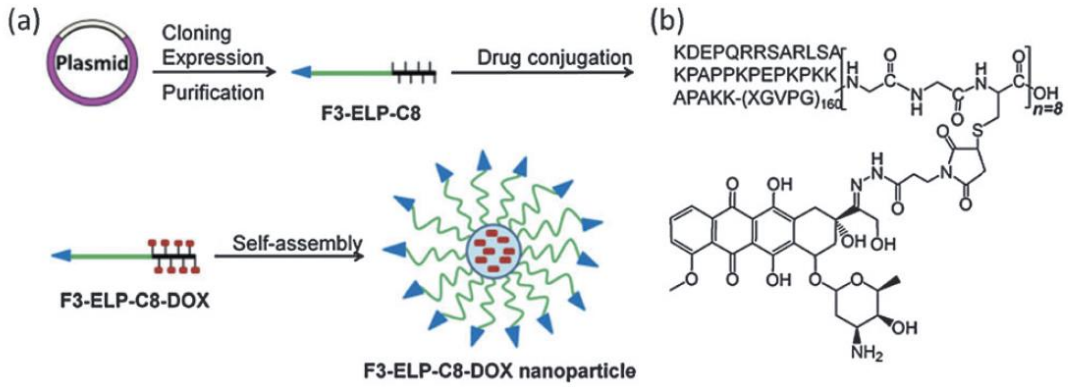

Figure 6. Schematic illustration of the synthesis of F3– ELR–C8–DOX nanoparticles. (a) The synthetic route of F3– ELR–C8–DOX nanoparticles. (b) The molecular structure of F3– ELR–C8–DOX. Adapted from reference [147]. Reproduced with permission from the RSC.

References

[1] E.M. Pridgen, F. Alexis, O.C. Farokhzad, Polymeric nanoparticle drug delivery technologies for oral delivery applications, Expert Opin Drug Deliv, 12 (2015) 1459-1473.

[2] C. Monge, J. Almodovar, T. Boudou, C. Picart, Spatio-Temporal Control of LbL Films for Biomedical Applications: From 2D to 3D, Advanced healthcare materials, 4 (2015) 811-830.

[3] D.E. Meyer, G.A. Kong, M.W. Dewhirst, M.R. Zalutsky, A. Chilkoti, Targeting a genetically engineered elastin-like polypeptide to solid tumors by local hyperthermia, Cancer research, 61 (2001) 1548-1554.

[4] J.C. Rodriguez-Cabello, A. Girotti, A. Ribeiro, F.J. Arias, Synthesis of genetically engineered protein polymers (recombinamers) as an example of advanced self-assembled smart materials, Methods in molecular biology (Clifton, N.J.), 811 (2012) 17-38.

[5] M.S. Desai, S.W. Lee, Protein-based functional nanomaterial design for bioengineering applications, Wiley interdisciplinary reviews. Nanomedicine and nanobiotechnology, 7 (2015) 69-97.

[6] M.B. van Eldijk, C.L. McGann, K.L. Kiick, J.C. van Hest, Elastomeric polypeptides, Top Curr Chem, 310 (2012) 71-116.

[7] R. Price, A. Poursaid, H. Ghandehari, Controlled release from recombinant polymers, J. Control Release, 190 (2014) 304-313.

[8] P. Shi, J.A. Gustafson, J.A. MacKay, Genetically engineered nanocarriers for drug delivery, International journal of nanomedicine, 9 (2014) 1617-1626.

[9] W. Huang, A. Rollett, D.L. Kaplan, Silk-elastin-like protein biomaterials for the controlled delivery of therapeutics, Expert Opin Drug Deliv, 12 (2015) 779-791.

[10] L. Li, T. Luo, K.L. Kiick, Temperature-triggered phase separation of a hydrophilic resilin-like polypeptide, Macromol Rapid Commun, 36 (2015) 90-95.

[11] T. Luo, L. He, P. Theato, K.L. Kiick, Thermoresponsive self-assembly of nanostructures from a collagen-like peptide-containing diblock copolymer, Macromolecular bioscience, 15 (2015) 111-123.

[12] S.M. Mithieux, A.S. Weiss, Elastin, Advances in Protein Chemistry, 70 (2005) 437-461.

[13] L. Duca, N. Floquet, A.J.P. Alix, B. Haye, L. Debelle, Elastin as a matrikine, Crit Rev Oncol Hemat, 49 (2004) 235-244.

[14] R.P. Mecham, A. Hinek, G.L. Griffin, R.M. Senior, L.A. Liotta, The elastin receptor shows structural and functional similarities to the 67-kDa tumor cell laminin receptor, J Biol Chem, 264 (1989) 16652-16657.

[15] D.V. Bax, U.R. Rodgers, M.M. Bilek, A.S. Weiss, Cell adhesion to tropoelastin is mediated via the C-terminal GRKRK motif and integrin alphaVbeta3, J Biol Chem, 284 (2009) 28616-28623.

[16] J.F. Almine, D.V. Bax, S.M. Mithieux, L. Nivison-Smith, J. Rnjak, A. Waterhouse, S.G. Wise, A.S. Weiss, Elastin-based materials, Chemical Society reviews, 39 (2010) 3371-3379.

[17] D.W. Urry, What sustains life? Consilient mechanisms for protein-based machines and materials, Springer-Verlag, New York, 2006.

[18] D.W. Urry, Molecular Machines: How Motion and Other Functions of Living Organisms Can Result from Reversible Chemical Changes, Angew. Chem., Int. Ed. Engl., 32 (1993) 819-841.

[19] P.L. San Biagio, F. Madonia, T.L. Trapane, D.W. Urry, The Overlap of Elastomeric Polypeptide Coils in Solution Required for Single-Phase Initiation of Elastogenesis, Chemical Physics Letters, 145 (1988) 571-574.
[20] D.W. Urry, Physical Chemistry of Biological Free Energy Transduction As Demonstrated by Elastic Protein-Based Polymers, Journal of Physical Chemistry B, 101 (1997) 11007-11028.
[21] J.C. Rodriguez-Cabello, M. Alonso, T. Perez, M.M. Herguedas, Differential scanning calorimetry study of the hydrophobic hydration of the elastin-based polypentapeptide, poly(VPGVG), from deficiency to excess of water, Biopolymers, 54 (2000) 282-288.
[22] C. Tanford, The hydrophobic effect: formation of micelles and biological membranes, Wiley, New York,, 1973.
[23] L. Pauling, R.E. Marsh, The Structure of Chlorine Hydrate, Proceedings of the National Academy of Sciences of the United States of America, 38 (1952) 112-118.
[24] D.W. Urry, T.L. Trapane, K.U. Prasad, Phase-structure transitions of the elastin polypentapeptide-water system within the framework of composition-temperature studies, Biopolymers, 24 (1985) 2345-2356.
[25] M. Manno, A. Emanuele, V. Martorana, P.L. San Biagio, D. Bulone, M.B. Palma-Vittorelli, D.T. McPherson, J. Xu, T.M. Parker, D.W. Urry, Interaction of processes on different length scales in a bioelastomer capable of performing energy conversion, Biopolymers, 59 (2001) 51-64.
[26] J.C. Rodríguez-Cabello, M. Alonso, L. Guiscardo, V. Reboto, A. Girotti, Amplified photoresponse of a p-phenylazobenzene derivative of an elastin-like polymer by alpha-cyclodextrin: The amplified Delta T-t mechanism, Advanced Materials, 14 (2002) 1151-1154.
[27] M. Alonso, V. Reboto, L. Guiscardo, A. San Martin, J.C. Rodríguez-Cabello, Spiropyran derivative of an elastin-like bioelastic polymer: Photoresponsive molecular machine to convert sunlight into mechanical work, Macromolecules, 33 (2000) 9480-9482.
[28] M. Alonso, V. Reboto, L. Guiscardo, V. Mate, J.C. Rodríguez-Cabello, Novel photoresponsive p-phenylazobenzene derivative of an elastin-like polymer with enhanced control of azobenzene content and without pH sensitiveness, Macromolecules, 34 (2001) 8072-8077.
[29] F. Ciardelli, D. Fabbri, O. Pieroni, A. Fissi, Photomodulation Of Polypeptide Conformation By Sunlight In Spiropyran-Containing Poly(L-Glutamic Acid), Journal of the American Chemical Society, 111 (1989) 3470-3472.
[30] D.W. Urry, T.M. Parker, M.C. Reid, D.C. Gowda, Biocompatibility of the Bioelastic Materials, Poly(GVGVP) and Its Gamma-Irradiation Cross-Linked Matrix - Summary of Generic Biological Test-Results, Journal of Bioactive and Compatible Polymers, 6 (1991) 263-282.
[31] S.R. Ong, K.A. Trabbic-Carlson, D.L. Nettles, D.W. Lim, A. Chilkoti, L.A. Setton, Epitope tagging for tracking elastin-like polypeptides, Biomaterials, 27 (2006) 1930-1935.
[32] C. García-Arévalo, J.F. Bermejo-Martín, L. Rico, V. Iglesias, L. Martín, J.C. Rodríguez-Cabello, F.J. Arias, Immunomodulatory Nanoparticles from Elastin-Like Recombinamers: Single-Molecules for Tuberculosis Vaccine Development, Molecular pharmaceutics, 10 (2013) 586-597.
[33] R.E. Sallach, W. Cui, F. Balderrama, A.W. Martinez, J. Wen, C.A. Haller, J.V. Taylor, E.R. Wright, R.C. Long, Jr., E.L. Chaikof, Long-term biostability of self-

assembling protein polymers in the absence of covalent crosslinking, Biomaterials, 31 (2010) 779-791.
[34] R. Herrero-Vanrell, A.C. Rincon, M. Alonso, V. Reboto, I.T. Molina-Martinez, J.C. Rodriguez-Cabello, Self-assembled particles of an elastin-like polymer as vehicles for controlled drug release, J Control Release, 102 (2005)113-22.
[35] A.J. Claxton, J. Cramer, C. Pierce, A systematic review of the associations between dose regimens and medication compliance, Clinical therapeutics, 23 (2001) 1296-1310.
[36] S. Mitragotri, P.A. Burke, R. Langer, Overcoming the challenges in administering biopharmaceuticals: formulation and delivery strategies, Nature reviews. Drug discovery, 13 (2014) 655-672.
[37] A. Hatefi, B. Amsden, Biodegradable injectable in situ forming drug delivery systems, J Control Release, 80 (2002) 9-28.
[38] S. Chaterji, I.K. Kwon, K. Park, Smart Polymeric Gels: Redefining the Limits of Biomedical Devices, Progress in polymer science, 32 (2007) 1083-1122.
[39] J. Kopecek, Polymer chemistry: swell gels, Nature, 417 (2002) 388-389, 391.
[40] M.C. Koetting, J.T. Peters, S.D. Steichen, N.A. Peppas, Stimulus-responsive hydrogels: Theory, modern advances, and applications, Materials Science and Engineering: R: Reports, 93 (2015) 1-49.
[41] J.E. Gagner, W. Kim, E.L. Chaikof, Designing protein-based biomaterials for medical applications, Acta biomaterialia, 10 (2014) 1542-1557.
[42] A.A. Dinerman, J. Cappello, H. Ghandehari, S.W. Hoag, Swelling behavior of a genetically engineered silk-elastinlike protein polymer hydrogel, Biomaterials, 23 (2002) 4203-4210.
[43] K. Trabbic-Carlson, L.A. Setton, A. Chilkoti, Swelling and mechanical behaviors of chemically cross-linked hydrogels of elastin-like polypeptides, Biomacromolecules, 4 (2003) 572-580.
[44] A.M. Testera, A. Girotti, I.G. de Torre, L. Quintanilla, M. Santos, M. Alonso, J.C. Rodriguez-Cabello, Biocompatible elastin-like click gels: design, synthesis and characterization, Journal of materials science. Materials in medicine, 26 (2015) 5435.
[45] N. Annabi, S.M. Mithieux, E.A. Boughton, A.J. Ruys, A.S. Weiss, F. Dehghani, Synthesis of highly porous crosslinked elastin hydrogels and their interaction with fibroblasts in vitro, Biomaterials, 30 (2009) 4550-4557.
[46] L. Martin, M. Alonso, A. Girotti, F.J. Arias, J.C. Rodriguez-Cabello, Synthesis and characterization of macroporous thermosensitive hydrogels from recombinant elastin-like polymers, Biomacromolecules, 10 (2009) 3015-3022.
[47] I. González de Torre, M. Santos, L. Quintanilla, A. Testera, M. Alonso, J.C. Rodríguez Cabello, Elastin-like recombinamer catalyst-free click gels: Characterization of poroelastic and intrinsic viscoelastic properties, Acta biomaterialia, 10 (2014) 2495-2505.
[48] Y.-N. Zhang, R.K. Avery, Q. Vallmajo-Martin, A. Assmann, A. Vegh, A. Memic, B.D. Olsen, N. Annabi, A. Khademhosseini, A Highly Elastic and Rapidly Crosslinkable Elastin-Like Polypeptide-Based Hydrogel for Biomedical Applications, Advanced Functional Materials, 25 (2015) 4814-4826.
[49] D.W. Urry, S.Q. Peng, T.M. Parker, Hydrophobicity-induced pK shifts in elastin protein-based polymers, Biopolymers, 32 (1992) 373-379.
[50] D.W. Urry, C.H. Luan, T.M. Parker, D.C. Gowda, K.U. Prasad, M.C. Reid, A. Safavy, Temperature of polypeptide inverse temperature transition depends on

mean residue hydrophobicity, Journal of the American Chemical Society, 113 (1991) 4346-4348.
[51] A. Ribeiro, F.J. Arias, J. Reguera, M. Alonso, J.C. Rodriguez-Cabello, Influence of the amino-acid sequence on the inverse temperature transition of elastin-like polymers, Biophys J, 97 (2009) 312-320.
[52] J. Reguera, D.W. Urry, T.M. Parker, D.T. McPherson, J.C. Rodriguez-Cabello, Effect of NaCl on the exothermic and endothermic components of the inverse temperature transition of a model elastin-like polymer, Biomacromolecules, 8 (2007) 354-358.
[53] J.C. Rodriguez-Cabello, V.E. Santo, S. Prieto, A.M. Testera, F.J. Arias, M. Alonso, J.F. Mano, Temperature-responsive bioactive hydrogels based on a multifunctional recombinant elastin-like polymer, Biomaterials and Biomedical Engineering, 2 (2015) 047.
[54] H. Betre, W. Liu, M.R. Zalutsky, A. Chilkoti, V.B. Kraus, L.A. Setton, A thermally responsive biopolymer for intra-articular drug delivery, J Control Release, 115 (2006) 175-182.
[55] S.R. MacEwan, A. Chilkoti, Applications of elastin-like polypeptides in drug delivery, J Control Release, 190 (2014) 314-330.
[56] A. Girotti, J. Reguera, J.C. Rodríguez-Cabello, F.J. Arias, M. Alonso, A.M. Testera, Design and bioproduction of a recombinant multi(bio)functional elastin-like protein polymer containing cell adhesion sequences for tissue engineering purposes, Journal of Materials Science-Materials in Medicine, 15 (2004) 479-484.
[57] A. Bandiera, A. Markulin, L. Corich, F. Vita, V. Borelli, Stimuli-induced release of compounds from elastin biomimetic matrix, Biomacromolecules, 15 (2014) 416-422.
[58] K. Straley, S.C. Heilshorn, Designer protein-based scaffolds for neural tissue engineering, Conf Proc IEEE Eng Med Biol Soc, 2009 (2009) 2101-2102.
[59] W. Wang, A. Jashnani, S.R. Aluri, J.A. Gustafson, P.Y. Hsueh, F. Yarber, R.L. McKown, G.W. Laurie, S.F. Hamm-Alvarez, J.A. MacKay, A thermo-responsive protein treatment for dry eyes, J Control Release, 199 (2015) 156-167.
[60] S.S. Amruthwar, A.V. Janorkar, Preparation and characterization of elastin-like polypeptide scaffolds for local delivery of antibiotics and proteins, Journal of materials science. Materials in medicine, 23 (2012) 2903-2912.
[61] W. Liu, J.A. MacKay, M.R. Dreher, M. Chen, J.R. McDaniel, A.J. Simnick, D.J. Callahan, M.R. Zalutsky, A. Chilkoti, Injectable intratumoral depot of thermally responsive polypeptide-radionuclide conjugates delays tumor progression in a mouse model, J Control Release, 144 (2010) 2-9.
[62] W. Liu, J. McDaniel, X. Li, D. Asai, F.G. Quiroz, J. Schaal, J.S. Park, M. Zalutsky, A. Chilkoti, Brachytherapy using injectable seeds that are self-assembled from genetically encoded polypeptides in situ, Cancer research, 72 (2012) 5956-5965.
[63] M. Amiram, K.M. Luginbuhl, X. Li, M.N. Feinglos, A. Chilkoti, Injectable protease-operated depots of glucagon-like peptide-1 provide extended and tunable glucose control, Proceedings of the National Academy of Sciences of the United States of America, 110 (2013) 2792-2797.
[64] M.F. Shamji, L. Whitlatch, A.H. Friedman, W.J. Richardson, A. Chilkoti, L.A. Setton, An injectable and in situ-gelling biopolymer for sustained drug release following perineural administration, Spine, 33 (2008) 748-754.
[65] S.M. Sinclair, J. Bhattacharyya, J.R. McDaniel, D.M. Gooden, R. Gopalaswamy, A. Chilkoti, L.A. Setton, A genetically engineered thermally responsive sustained

release curcumin depot to treat neuroinflammation, J Control Release, 171 (2013) 38-47.
[66] M.F. Shamji, L. Jing, J. Chen, P. Hwang, O. Ghodsizadeh, A.H. Friedman, W.J. Richardson, L.A. Setton, Treatment of neuroinflammation by soluble tumor necrosis factor receptor Type II fused to a thermally responsive carrier, Journal of neurosurgery. Spine, 9 (2008) 221-228.
[67] I. Uckay, P. Hoffmeyer, D. Lew, D. Pittet, Prevention of surgical site infections in orthopaedic surgery and bone trauma: state-of-the-art update, J Hosp Infect, 84 (2013) 5-12.
[68] S.B. Adams, M.F. Shamji, D.L. Nettles, P. Hwang, L.A. Setton, Sustained release of antibiotics from injectable and thermally responsive polypeptide depots, Journal of Biomedical Materials Research Part B: Applied Biomaterials, 90B (2009) 67-74.
[69] T.R. Anderson, M.E. Marquart, A.V. Janorkar, Effective release of a broad spectrum antibiotic from elastin-like polypeptide-collagen composite, Journal of biomedical materials research. Part A, 103 (2015) 782-790.
[70] N.J. Friedman, Impact of dry eye disease and treatment on quality of life, Current opinion in ophthalmology, 21 (2010) 310-316.
[71] T. Vijmasi, F.Y. Chen, S. Balasubbu, M. Gallup, R.L. McKown, G.W. Laurie, N.A. McNamara, Topical administration of lacritin is a novel therapy for aqueous-deficient dry eye disease, Investigative ophthalmology & visual science, 55 (2014) 5401-5409.
[72] M. Bigoni, P. Sacerdote, M. Turati, S. Franchi, M. Gandolla, D. Gaddi, S. Moretti, D. Munegato, C.A. Augusti, E. Bresciani, R.J. Omeljaniuk, V. Locatelli, A. Torsello, Acute and late changes in intraarticular cytokine levels following anterior cruciate ligament injury, J Orthopaedic Research, 31 (2013) 315-321.
[73] M.F. Shamji, H. Betre, V.B. Kraus, J. Chen, A. Chilkoti, R. Pichika, K. Masuda, L.A. Setton, 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 and rheumatism, 56 (2007) 3650-3661.
[74] K.D. Allen, S.B. Adams, Jr., B.A. Mata, M.F. Shamji, E. Gouze, L. Jing, D.L. Nettles, L.D. Latt, L.A. Setton, Gait and behavior in an IL1beta-mediated model of rat knee arthritis and effects of an IL1 antagonist, J Orthopaedic Research, 29 (2011) 694-703.
[75] M.F. Shamji, J. Chen, A.H. Friedman, W.J. Richardson, A. Chilkoti, L.A. Setton, Synthesis and characterization of a thermally-responsive tumor necrosis factor antagonist, J Control Release, 129 (2008) 179-186.
[76] K.A. Kimmerling, B.D. Furman, D.S. Mangiapani, M.A. Moverman, S.M. Sinclair, J.L. Huebner, A. Chilkoti, V.B. Kraus, L.A. Setton, F. Guilak, S.A. Olson, Sustained intra-articular delivery of IL-1RA from a thermally-responsive elastin-like polypeptide as a therapy for post-traumatic arthritis, European cells & materials, 29 (2015) 124-139; discussion 139-140.
[77] L. Prasad-Reddy, D. Isaacs, A clinical review of GLP-1 receptor agonists: efficacy and safety in diabetes and beyond, Drugs in context, 4 (2015) 212283.
[78] H. Poudyal, Mechanisms for the Cardiovascular Effects of Glucagon-Like Peptide-1, Acta Physiol (Oxf), (2015). doi: 10.1111/apha.12604.
[79] M. Amiram, K.M. Luginbuhl, X. Li, M.N. Feinglos, A. Chilkoti, A depot-forming glucagon-like peptide-1 fusion protein reduces blood glucose for five days with a single injection, J Control Release, 172 (2013) 144-151.

[80] J.A. Hubbell, A. Chilkoti, Chemistry. Nanomaterials for drug delivery, Science, 337 (2012) 303-305.
[81] H. Zou, Z. Wang, M. Feng, Nanocarriers with tunable surface properties to unblock bottlenecks in systemic drug and gene delivery, J Control Release, 214 (2015) 121-133.
[82] Y. Zhang, H.F. Chan, K.W. Leong, Advanced materials and processing for drug delivery: the past and the future, Advanced drug delivery reviews, 65 (2013) 104-120.
[83] S. Ganta, H. Devalapally, A. Shahiwala, M. Amiji, A review of stimuli-responsive nanocarriers for drug and gene delivery, J. Control Release, 126 (2008) 187-204.
[84] D.S. Spencer, A.S. Puranik, N.A. Peppas, Intelligent Nanoparticles for Advanced Drug Delivery in Cancer Treatment, Current opinion in chemical engineering, 7 (2015) 84-92.
[85] R. Lin, H. Cui, Supramolecular nanostructures as drug carriers, Current opinion in chemical engineering, 7 (2015) 75-83.
[86] A. Girotti, D. Orbanic, A. Ibanez-Fonseca, C. Gonzalez-Obeso, J.C. Rodriguez-Cabello, Recombinant Technology in the Development of Materials and Systems for Soft-Tissue Repair, Advanced healthcare materials, , 4 (2015) 2423-2455.
[87] S.M. Janib, M. Pastuszka, S. Aluri, Z. Folchman-Wagner, P.Y. Hsueh, P. Shi, A. Yi, H. Cui, J.A. Mackay, A quantitative recipe for engineering protein polymer nanoparticles, Polymer chemistry, 5 (2014) 1614-1625.
[88] A.J. Simnick, M. Amiram, W. Liu, G. Hanna, M.W. Dewhirst, C.D. Kontos, A. Chilkoti, In vivo tumor targeting by a NGR-decorated micelle of a recombinant diblock copolypeptide, J Control Release, 155 (2011) 144-151.
[89] L. Martín, E. Castro, A. Ribeiro, M. Alonso, J.C. Rodríguez-Cabello, Temperature-Triggered Self-Assembly of Elastin-Like Block Co-Recombinamers:The Controlled Formation of Micelles and Vesicles in an Aqueous Medium, Biomacromolecules, 13 (2012) 293-298.
[90] J.R. McDaniel, I. Weitzhandler, S. Prevost, K.B. Vargo, M.S. Appavou, D.A. Hammer, M. Gradzielski, A. Chilkoti, Noncanonical self-assembly of highly asymmetric genetically encoded polypeptide amphiphiles into cylindrical micelles, Nano letters, 14 (2014) 6590-6598.
[91] W.M. Park, J.A. Champion, Thermally triggered self-assembly of folded proteins into vesicles, Journal of the American Chemical Society, 136 (2014) 17906-17909.
[92] D.J. Callahan, W. Liu, X. Li, M.R. Dreher, W. Hassouneh, M. Kim, P. Marszalek, A. Chilkoti, Triple stimulus-responsive polypeptide nanoparticles that enhance intratumoral spatial distribution, Nano letters, 12 (2012) 2165-2170.
[93] M. Shah, M.C. Edman, S.R. Janga, P. Shi, J. Dhandhukia, S. Liu, S.G. Louie, K. Rodgers, J.A. Mackay, S.F. Hamm-Alvarez, A rapamycin-binding protein polymer nanoparticle shows potent therapeutic activity in suppressing autoimmune dacryoadenitis in a mouse model of Sjogren's syndrome, J Control Release, 171 (2013) 269-279.
[94] P. Shi, S. Aluri, Y.A. Lin, M. Shah, M. Edman, J. Dhandhukia, H. Cui, J.A. MacKay, Elastin-based protein polymer nanoparticles carrying drug at both corona and core suppress tumor growth in vivo, J Control Release, 171 (2013) 330-338.
[95] G. Sun, P.-Y. Hsueh, S.M. Janib, S. Hamm-Alvarez, J. Andrew MacKay, Design and cellular internalization of genetically engineered polypeptide nanoparticles displaying adenovirus knob domain, J Control Release, 155 (2011) 218-226.

[96] P.Y. Hsueh, M.C. Edman, G. Sun, P. Shi, S. Xu, Y.A. Lin, H. Cui, S.F. Hamm-Alvarez, J.A. MacKay, Tear-mediated delivery of nanoparticles through transcytosis of the lacrimal gland, J Control Release, 208 (2015) 2-13.
[97] R. Machado, P.C. Bessa, R.L. Reis, J.C. Rodriguez-Cabello, M. Casal, Elastin-based nanoparticles for delivery of bone morphogenetic proteins, Methods in molecular biology, 906 (2012) 353-363.
[98] P.C. Bessa, R. Machado, S. Nürnberger, D. Dopler, A. Banerjee, A.M. Cunha, J.C. Rodríguez-Cabello, H. Redl, M. van Griensven, R.L. Reis, M. Casal, Thermoresponsive self-assembled elastin-based nanoparticles for delivery of BMPs, J Control Release, 142 (2010) 312-318.
[99] R.R. Costa, C.A. Custodio, F.J. Arias, J.C. Rodriguez-Cabello, J.F. Mano, Nanostructured and thermoresponsive recombinant biopolymer-based microcapsules for the delivery of active molecules, Nanomedicine, 9 (2013) 895-902.
[100] R.R. Costa, A. Girotti, M. Santos, F.J. Arias, J.F. Mano, J.C. Rodriguez-Cabello, Cellular uptake of multilayered capsules produced with natural and genetically engineered biomimetic macromolecules, Acta biomaterialia, 10 (2014) 2653-2662.
[101] P. Koria, H. Yagi, Y. Kitagawa, Z. Megeed, Y. Nahmias, R. Sheridan, M.L. Yarmush, Self-assembling elastin-like peptides growth factor chimeric nanoparticles for the treatment of chronic wounds, Proceedings of the National Academy of Sciences of the United States of America, 108 (2011) 1034-1039.
[102] Y. Assal, Y. Mizuguchi, M. Mie, E. Kobatake, Growth Factor Tethering to Protein Nanoparticles via Coiled-Coil Formation for Targeted Drug Delivery, Bioconjugate chemistry, 26 (2015) 1672-1677.
[103] G.L. Bidwell, 3rd, D. Raucher, Cell penetrating elastin-like polypeptides for therapeutic peptide delivery, Advanced drug delivery reviews, 62 (2010) 1486-1496.
[104] L.R. Walker, J.S. Ryu, E. Perkins, L.R. McNally, D. Raucher, Fusion of cell-penetrating peptides to thermally responsive biopolymer improves tumor accumulation of p21 peptide in a mouse model of pancreatic cancer, Drug design, development and therapy, 8 (2014) 1649-1658.
[105] S.R. MacEwan, A. Chilkoti, Controlled apoptosis by a thermally toggled nanoscale amplifier of cellular uptake, Nano letters, 14 (2014) 2058-2064.
[106] K.K. Krishnani, W. Chen, A. Mulchandani, Bactericidal activity of elastin-like polypeptide biopolymer with polyhistidine domain and silver, Colloids and surfaces. B, Biointerfaces, 119 (2014) 66-70.
[107] D. Raucher, J.S. Ryu, Cell-penetrating peptides: strategies for anticancer treatment, Trends in molecular medicine, 21 (2015) 560-570.
[108] J.S. Ryu, D. Raucher, Anti-tumor efficacy of a therapeutic peptide based on thermo-responsive elastin-like polypeptide in combination with gemcitabine, Cancer letters, 348 (2014) 177-184.
[109] J.S. Ryu, M. Kuna, D. Raucher, Penetrating the cell membrane, thermal targeting and novel anticancer drugs: the development of thermally targeted, elastin-like polypeptide cancer therapeutics, Therapeutic delivery, 5 (2014) 429-445.
[110] A. da Costa, R. Machado, A. Ribeiro, T. Collins, V. Thiagarajan, M.T. Neves-Petersen, J.C. Rodriguez-Cabello, A.C. Gomes, M. Casal, Development of elastin-like recombinamer films with antimicrobial activity, Biomacromolecules, 16 (2015) 625-635.

[111] M.R. Dreher, W. Liu, C.R. Michelich, M.W. Dewhirst, A. Chilkoti, Thermal cycling enhances the accumulation of a temperature-sensitive biopolymer in solid tumors, Cancer research, 67 (2007) 4418-4424.
[112] E.M. George, H. Liu, G.G. Robinson, G.L. Bidwell, A polypeptide drug carrier for maternal delivery and prevention of fetal exposure, Journal of drug targeting, 22 (2014) 935-947.
[113] W. Liu, M.R. Dreher, D.C. Chow, M.R. Zalutsky, A. Chilkoti, Tracking the in vivo fate of recombinant polypeptides by isotopic labeling, J Control Release, 114 (2006) 184-192.
[114] F.S. Nouri, X. Wang, X. Chen, A. Hatefi, Reducing the Visibility of the Vector/DNA Nanocomplexes to the Immune System by Elastin-Like Peptides, Pharmaceutical research, 32 (2015) 3018-3028.
[115] M. Shah, P.Y. Hsueh, G. Sun, H.Y. Chang, S.M. Janib, J.A. MacKay, Biodegradation of elastin-like polypeptide nanoparticles, Protein science, 21 (2012) 743-750.
[116] T.-H. Chen, Y. Bae, D. Furgeson, Intelligent Biosynthetic Nanobiomaterials (IBNs) for Hyperthermic Gene Delivery, Pharmaceutical research, 25 (2008) 683-691.
[117] M. Piña, S. Alex, F. Arias, M. Santos, J. Rodriguez-Cabello, R. Ramesan, C. Sharma, Elastin-like recombinamers with acquired functionalities for gene-delivery applications, Journal of biomedical materials research. Part A, 103 (2015) 3166-3178.
[118] F.C. Smits, B.C. Buddingh, M.B. van Eldijk, J.C. van Hest, Elastin-like polypeptide based nanoparticles: design rationale toward nanomedicine, Macromolecular bioscience, 15 (2015) 36-51.
[119] E.M. Mastria, M. Chen, J.R. McDaniel, X. Li, J. Hyun, M.W. Dewhirst, A. Chilkoti, Doxorubicin-conjugated polypeptide nanoparticles inhibit metastasis in two murine models of carcinoma, J Control Release, 208 (2015) 52-58.
[120] P. Zhao, S. Dong, J. Bhattacharyya, M. Chen, iTEP nanoparticle-delivered salinomycin displays an enhanced toxicity to cancer stem cells in orthotopic breast tumors, Molecular pharmaceutics, 11 (2014) 2703-2712.
[121] J. Bhattacharyya, J.J. Bellucci, I. Weitzhandler, J.R. McDaniel, I. Spasojevic, X. Li, C.-C. Lin, J.-T.A. Chi, A. Chilkoti, A paclitaxel-loaded recombinant polypeptide nanoparticle outperforms Abraxane in multiple murine cancer models, Nature communications, 6 (2015) 7939.
[122] J.R. McDaniel, J. Bhattacharyya, K.B. Vargo, W. Hassouneh, D.A. Hammer, A. Chilkoti, Self-assembly of thermally responsive nanoparticles of a genetically encoded peptide polymer by drug conjugation, Angewandte Chemie, 52 (2013) 1683-1687.
[123] J.R. McDaniel, S.R. MacEwan, X. Li, D.C. Radford, C.D. Landon, M. Dewhirst, A. Chilkoti, Rational design of "heat seeking" drug loaded polypeptide nanoparticles that thermally target solid tumors, Nano letters, 14 (2014) 2890-2895.
[124] C. Vepari, D.L. Kaplan, Silk as a Biomaterial, Progress in polymer science, 32 (2007) 991-1007.
[125] Z. Zhao, Y. Li, M.B. Xie, Silk fibroin-based nanoparticles for drug delivery, International journal of molecular sciences, 16 (2015) 4880-4903.
[126] K. Numata, J. Hamasaki, B. Subramanian, D.L. Kaplan, Gene delivery mediated by recombinant silk proteins containing cationic and cell binding motifs, J Control Release 146 (2010) 136-143.

[127] M. Hofer, G. Winter, J. Myschik, Recombinant spider silk particles for controlled delivery of protein drugs, Biomaterials, 33 (2012) 1554-1562.
[128] J.A. Gustafson, H. Ghandehari, Silk-elastinlike protein polymers for matrix-mediated cancer gene therapy, Advanced drug delivery reviews, 62 (2010) 1509-1523.
[129] J.L. Frandsen, H. Ghandehari, Recombinant protein-based polymers for advanced drug delivery, Chemical Society reviews, 41 (2012) 2696-2706.
[130] R. Anumolu, J.A. Gustafson, J.J. Magda, J. Cappello, H. Ghandehari, L.F. Pease, 3rd, Fabrication of highly uniform nanoparticles from recombinant silk-elastin-like protein polymers for therapeutic agent delivery, ACS nano, 5 (2011) 5374-5382.
[131] X.-X. Xia, Q. Xu, X. Hu, G. Qin, D.L. Kaplan, Tunable Self-Assembly of Genetically Engineered Silk-Elastin-like Protein Polymers, Biomacromolecules, 12 (2011) 3844-3850.
[132] X.X. Xia, M. Wang, Y. Lin, Q. Xu, D.L. Kaplan, Hydrophobic drug-triggered self-assembly of nanoparticles from silk-elastin-like protein polymers for drug delivery, Biomacromolecules, 15 (2014) 908-914.
[133] A. Fernandez-Colino, F.J. Arias, M. Alonso, J.C. Rodriguez-Cabello, Self-organized ECM-mimetic model based on an amphiphilic multiblock silk-elastin-like corecombinamer with a concomitant dual physical gelation process, Biomacromolecules, 15 (2014) 3781-3793.
[134] R.R. Costa, E. Castro, F. Javier Arias, J. Carlos Rodriguez-Cabello, J.F. Mano, Multifunctional Compartmentalized Capsules with a Hierarchical Organization from the Nano to the Macro Scales, Biomacromolecules, 14 (2013) 2403-2410.
[135] S.M. Park, J.M. Cha, J. Nam, M.S. Kim, S.J. Park, E.S. Park, H. Lee, H.R. Kim, Formulation optimization and in vivo proof-of-concept study of thermosensitive liposomes balanced by phospholipid, elastin-like polypeptide, and cholesterol, PloS one, 9 (2014) e103116.
[136] W. Han, S.R. MacEwan, A. Chilkoti, G.P. Lopez, Bio-inspired synthesis of hybrid silica nanoparticles templated from elastin-like polypeptide micelles, Nanoscale, 7 (2015) 12038-12044.
[137] D. Fukushima, U.H. Sk, Y. Sakamoto, I. Nakase, C. Kojima, Dual stimuli-sensitive dendrimers: Photothermogenic gold nanoparticle-loaded thermo-responsive elastin-mimetic dendrimers, Colloids and surfaces. B, Biointerfaces, 132 (2015) 155-160.
[138] J.C. Rodríguez-Cabello, L. Martín, M. Alonso, F.J. Arias, A.M. Testera, "Recombinamers" as advanced materials for the post-oil age, Polymer, 50 (2009) 5159-5169.
[139] D.Y. Furgeson, M.R. Dreher, A. Chilkoti, Structural optimization of a "smart" doxorubicin-polypeptide conjugate for thermally targeted delivery to solid tumors, J Control Release, 110 (2006) 362-369.
[140] M.R. Dreher, D. Raucher, N. Balu, O. Michael Colvin, S.M. Ludeman, A. Chilkoti, Evaluation of an elastin-like polypeptide-doxorubicin conjugate for cancer therapy, J Control Release, 91 (2003) 31-43.
[141] G.L. Bidwell, 3rd, A.N. Davis, I. Fokt, W. Priebe, D. Raucher, A thermally targeted elastin-like polypeptide-doxorubicin conjugate overcomes drug resistance, Investigational new drugs, 25 (2007) 313-326.
[142] I. Massodi, G.L. Bidwell Iii, D. Raucher, Evaluation of cell penetrating peptides fused to elastin-like polypeptide for drug delivery, J Control Release, 108 (2005) 396-408.

[143] S. Moktan, C. Ryppa, F. Kratz, D. Raucher, A thermally responsive biopolymer conjugated to an acid-sensitive derivative of paclitaxel stabilizes microtubules, arrests cell cycle, and induces apoptosis, Investigational new drugs, 30 (2012) 236-248.
[144] C. Rousselle, M. Smirnova, P. Clair, J.M. Lefauconnier, A. Chavanieu, B. Calas, J.M. Scherrmann, J. Temsamani, Enhanced delivery of doxorubicin into the brain via a peptide-vector-mediated strategy: saturation kinetics and specificity, J Pharmacol Exp Ther, 296 (2001) 124-131.
[145] S. Moktan, E. Perkins, F. Kratz, D. Raucher, Thermal targeting of an acid-sensitive doxorubicin conjugate of elastin-like polypeptide enhances the therapeutic efficacy compared with the parent compound in vivo, Molecular cancer therapeutics, 11 (2012) 1547-1556.
[146] J.A. MacKay, M. Chen, J.R. McDaniel, W. Liu, A.J. Simnick, A. Chilkoti, Self-assembling chimeric polypeptide-doxorubicin conjugate nanoparticles that abolish tumours after a single injection, Nature materials, 8 (2009) 993-999.
[147] J. Hu, L. Xie, W. Zhao, M. Sun, X. Liu, W. Gao, Design of tumor-homing and pH-responsive polypeptide-doxorubicin nanoparticles with enhanced anticancer efficacy and reduced side effects, Chemical communications, 51 (2015) 11405-11408.
[148] W. Wang, J. Despanie, P. Shi, M.C. Edman-Woolcott, Y.A. Lin, H. Cui, J.M. Heur, M.E. Fini, S.F. Hamm-Alvarez, J.A. MacKay, Lacritin-mediated regeneration of the corneal epithelia by protein polymer nanoparticles, Journal of materials chemistry. B, Materials for biology and medicine, 2 (2014) 8131-8141.
[149] C.L.d. Rio, R. George, P. Kloepfer, Y. Ueyama, B. Youngblood, L. Georgopoulos, S. Arnold, R.L. Hamlin, VASOMERA™, A Novel Vpac2-Selective Vasoactive Intestinal Peptide Agonist, Enhances Contractility And Decreases Myocardial Demand In Dogs With Both Normal Hearts And With Pacing-Induced Dilated Cardiomyopathy, Journal of the American College of Cardiology, 61 (2013).
[150] R. Price, A. Poursaid, J. Cappello, H. Ghandehari, In vivo evaluation of matrix metalloproteinase responsive silk-elastinlike protein polymers for cancer gene therapy, J Control Release, 213 (2015) 96-102.
[151] B.C. Dash, D. Thomas, M. Monaghan, O. Carroll, X. Chen, K. Woodhouse, T. O'Brien, A. Pandit, An injectable elastin-based gene delivery platform for dose-dependent modulation of angiogenesis and inflammation for critical limb ischemia, Biomaterials, 65 (2015) 126-139.