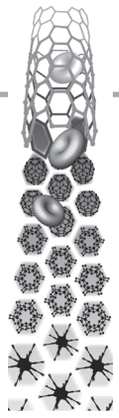

Emerging applications of multifunctional elastin-like recombinamers

Elastin-like recombinamers have grown in popularity in the field of protein-inspired biomimetic materials and have found widespread use in biomedical applications. Modern genetic-engineering techniques have allowed the design of multifunctional materials with an extraordinary control over their architecture and physicochemical properties, such as stimuli-responsiveness, monodispersity, biocompatibility or self-assembly, amongst others. Indeed, these materials are playing an increasingly important role in a diverse range of applications, such as drug delivery, tissue engineering and 'smart' systems. Herein, we review some of the most interesting examples of recent advances and progressive applications of elastin-like recombinamers in the biomaterial and nano-engineering sciences in recent years.

KEYWORDS: drug delivery ▪ elastin-like recombinamers ▪ nano-devices ▪ protein purification ▪ surface engineering ▪ tissue engineering

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As a result of their unique and specific interactions with other macromolecules and inorganic compounds, proteins are an indispensable part of biological structures and systems, especially as regards control of tissue formation, biological functions or physical performance [1]. Nanobiotechnological approaches focused on genetically engineered, protein-based biopolymers have been considered to show great promise for the development of advanced biomaterials with applications in medicine-related fields and nanotechnology owing to their valuable properties, such as their propensity to form hydrophilic networks, their expected good biocompatibility, favorable degradation rate and products, very low cytotoxicity and immunogenicity, and the ability to design structurally complex constructs with tunable mechanical, physical or biological properties [2,3]. Indeed, considerable progress has been made as regards the use of these protein-based biomaterials in recent years, with elastin-like polypeptides being one of the most studied protein-like structures [4–6].

Elastin is an insoluble elastic protein that dominates flexible tissues where elasticity is of major importance (e.g., skin, ligament, arteries, lungs and specialized cartilages). The increasing availability of recombinant forms of elastin that are virtually invisible to the immune system has allowed the formation of a wide range of biomaterial constructs and composites that benefit from elastin's inherent properties of innate assembly and elasticity [4]. The term elastin-like recombinamers (ELRs) highlights the fact that these constructs are oligomeric macromolecules whose composition is strictly defined by engineering design, that they are produced as recombinant proteins that exhibit monodispersity and a high control over amino-acid sequence, and that they mimic the basic properties of elastin [7]. ELRs, which are made of repeating amino-acid sequences from the five-member unit (VPGX$_{aa}$G), where X$_{aa}$ is any natural amino-acid except proline, or its permutations, can be tailor-made to form self-assembled systems with controlled structures and functions [8]. The hydrophobic, clathrate-like, hydrated chains of ELRs break above a characteristic transition temperature (Tt), thereby allowing ELRs to fold hydrophobically to form a separated state in which the polymer chains adopt a dynamic, regular, nonrandom structure known as a β-spiral. This structure contains type II β-turns as the main secondary feature and is stabilized by intraspiral, interturn, and interspiral hydrophobic contacts. This process starts with the formation of filaments composed of three-stranded dynamic polypeptide β-spirals, which grow to form particles several hundred nanometers in diameter before settling into a visibly separate state, and is completely reversible upon lowering the sample temperature below T$_t$ [9]. Although this β-spiral model is the structure that appears to best account for the available experimental data, there is still some controversy regarding its actual significance. Indeed, some studies have shown that other conformations are present in elastin (crosslinking and plastic domains) [10]. These observations include X-ray crystallography, solution and solid-state nuclear

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magnetic resonance (NMR) [11] spectroscopic studies and molecular dynamics simulations [12,13]. However, in this kind of elastomeric ELR, where large amounts of water play an active role in increasing the dynamics of the polymer chain, it is not surprising that different methodological techniques, which observe the ELR chain on different time scales, give rise to apparently contradictory results.

The characteristic and reversible coacervation exhibited by ELRs in response to temperature changes in an aqueous environment can be a complex process that is strongly influenced by the composition of the ELRs but also depends on the molecular mass, the degree of ionization of any functional side chains, the mean polarity of the polymer, salt concentration and the presence of other ions and molecules [7].

The control of nanostructures and the ordered 2D and 3D assembly of materials into useful functional structures and devices is one of the main challenges in the development of novel engineered biomaterials. The ability of ELR-based systems to display stimuli-driven variations in their nanostructures as a consequence of their self-assembling behavior in aqueous solution has been exploited in the development of complex 'smart' biomaterials in which the combination of specific building blocks has led to a wide range of sizes, morphologies and functional possibilities. In addition, the recombinant route allows such polymers to be designed with a view to their intended use by taking advantage of the superior and easy sequence-control possibilities offered by this method, hence their physicochemical and mechanical properties can be fine-tuned to meet the desired applications (Figure 1).

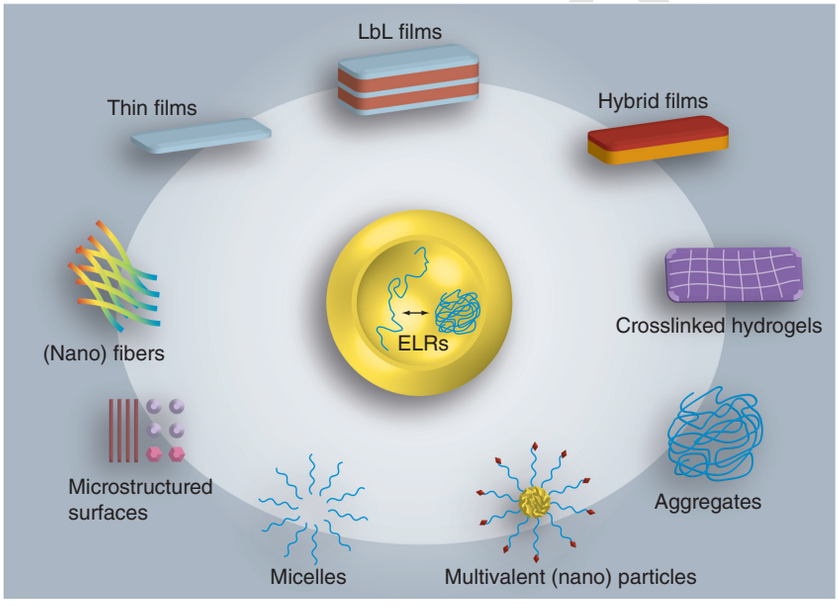

Figure 1. Summary of some applications of elastin-like recombinamers. Different ELR-based devices, including aggregates, films (thin films or membranes of polymer networks, self-assembled LbL films or hybrid systems combining different polymers and particles), physically or chemically crosslinked hydrogels and nanofibers, and nanoparticles, such as micelles or multivalent nanoparticles, that assemble in solution have been developed for use in various applications (e.g., drug delivery, tissue engineering, cell harvesting, smart and bioactive surfaces, and protein purification). ELR: Elastin-like recombinamer; LbL: Layer-by-layer.

Applications

Tissue engineering

Owing to the fact that they are biomedical polymers, ELRs have found widespread use due to their ability to remain essentially invisible to the host immune system in a large spectrum of applications, mainly in the fields of tissue engineering and drug delivery. Indeed, the immune system appears to ignore these polymers and their degradation products (amino acids) as it is unable to distinguish them from natural elastin [14]. Scaffolds for either *in vitro* cultivation and subsequent transplantation or *in vivo* implantation directly into the body to support or replace specific biological or physiological functions (e.g., tissue repair or tissue engineering) should be colonized by cells that proliferate and secrete extracellular matrix (ECM) and growth factors and must be suitable for the diffusion of nutrients, oxygen and waste from these cells until the cells themselves are able to support the defective organ, thus replacing the implanted scaffold and maintaining healthy cell development and functionality [15]. Thus, aspects related to both the topology and structure of the scaffolds and their bioactivity are crucial for the subsequent fate of these cell-supporting systems. However, finding the ideal combination of a biomaterial and cells that provide suitable environmental conditions to form a functional biological system resembling the physiological one is a key challenge when designing such constructs. In this regard, ELRs are biocompatible starting materials that can be provided with the appropriate mechanical properties and lack nonspecific bioactivities that could promote nonspecific cell adhesion [16]. The antifouling properties and biocompatibility of simple ELRs and their cross-linked matrices have been reported [17,18]. Likewise, recombinant human elastin polypeptide fragments in polymers have been shown to be an efficient coating on synthetic materials, demonstrating reduced platelet activation and adhesion in platelet-rich plasma *in vitro* and therefore great potential as a nonthrombogenic biomaterial [19]. These simple molecules were subsequently enriched with

short peptides to provide specific bioactivities. To increase the complexity of ELRs, a simple substitution of the amino acid X in the elastin-based repeat unit (VPGXG) can result in the formation of cross-linking domains and thus more uniform and stable substrates. Thus, two- and 3D cross-linked scaffolds with a lysine in the X position, which can be used in both enzymatic and chemical reactions for conjugation or cross-linking purposes, have been reported [20–22]. Likewise, ELR hydrogels have been produced by photoinitiation [23] or irradiation [24].

ELRs are able to form stable viscoelastic hydrogels with controlled mechanical and physico-structural features that maintain their interesting properties as stimuli-responsive substrates. Tirrell and coworkers have demonstrated that chemically cross-linked hydrogels formed in organic solvents from ELRs containing fibronectin cell-binding domains can successfully be used to support the growth and spread of endothelial cells [25,26]. ELR hydrogels have also demonstrated the ability to induce the differentiation of adult human adipose stem cells into the chondrocytic phenotype and the accumulation of cartilage-specific ECM in the absence of exogenous chondrogenic supplements *in vitro*, thereby potentially simplifying and reducing the cost of the differentiation process [27]. The chemical gelation of ELRs has also been performed in aqueous solution. Thus, Lim *et al.* have reported the formation of hydrogels with controlled mechanical properties in a biocompatible and injectable biomaterial for supporting tissue regeneration under physiological conditions [28]. Likewise, we have reported the production of 3D-ELR structured hydrogels with controlled microtopography (lines or pillars, squares, hexagons and so on) and different sizes and depths by replica molding. The resulting scaffolds showed a controlled microtopography with modulated mechanical properties and stimuli-responsive behavior in aqueous media. These hydrogels may find a use in the study and control of cell behavior by controlling their topography. Furthermore, the smart nature of these gels makes the substrates active, with the possibility of changing the dimensions of the microstructures during cell culture, provided the Tt is adjusted in the correct range [21].

Another alternative and very attractive approach, whereby some ELRs are able to form stable matrices under mild, physiological conditions based on their innate temperature responsiveness, has been shown to be effective for the formation of ELR hydrogels in aqueous solution. Temperature-responsive polymers are very promising base materials for '*in situ*-generated implants' owing to their ability to form low viscosity physiological solutions at room temperature, which form gels at higher temperatures [29]. The modular structure of ELRs allows the design and incorporation of specific features to enhance the self-assembly behavior, thereby resulting in physically cross-linked ELR hydrogels that are suitable for replacing soft tissue but lack the strength required for some other tissue-engineering applications [18]. Sallach *et al.* have also developed a recombinant elastin-mimetic triblock-copolymer in the absence of either chemical or ionic cross-linking that shows a minimal inflammatory response and robust *in vivo* stability for periods exceeding 1 year, thus further highlighting the high and extraordinary biocompatibility of ELRs. These triblock copolymers could also be used as structural components of artificial organs and engineered living tissues, as carriers for controlled drug release, or as biocompatible surface coatings [30]. Likewise, Martín *et al.* have demonstrated a simple, fast, water-based method to obtain highly resolved micropatterns by biocompatible replica molding involving a more complex optimized ELR amphiphilic elastin-like tetrablock-copolymer (FIGURE 2). This process involves a one-step process in which the aqueous ELP solution is simply heated slightly above its gelation temperature (Tgel) to obtain micropatterned biocompatible gels [29]. The aim of this work was to demonstrate the processability of ELRs by replica molding to obtain different systems that mimic *in vivo* cellular environments, thereby enabling future *in vitro* studies of cell matrix interactions. There are currently no generally accepted trends in terms of cell behavior control owing to differences in the numerous parameters involved, especially cell type, substrate stiffness and length scale features, amongst others [31]. A wide range of different geometries and topographies, with topographical features ranging from 10–100 µm in width, have been prepared. The step height of the micro-features was 15 µm for the pits and grooves and 9 µm for the pillars. In addition to the possibility to obtain multibioactive versions of these self-gelating polymers with different functionalities, such as interactions with cells or others, this physical gelation approach makes these polymers excellent substrates to emulate different tissues and as a proving ground for many studies dealing with cell–material interactions.

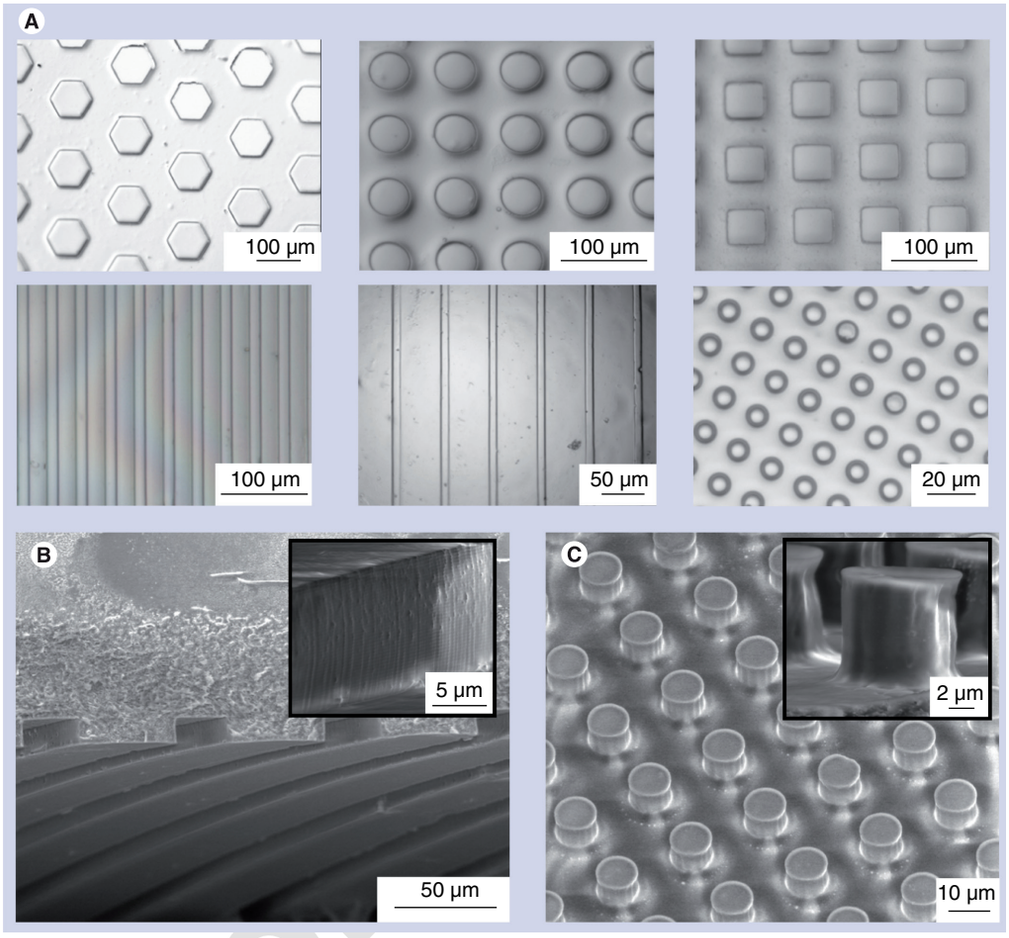

Figure 2. Micropatterned gels with different features obtained by replica molding.
(A) Optical differential interference contrast micrographs of swollen gels in phosphate buffered saline. **(B & C)** Environmental scanning electron microscope micrographs.
Reproduced with permission from [29].

Diverse synthetic materials functionalized by the integration of extracellular adhesion ligands and growth factors have demonstrated their effectiveness in directing cell-material signaling [32]. Selected amino acid domains can also be incorporated into ELR monomer sequences with the aim of recreating the mechanical and physiological features of ECM proteins. This strategy allows the synthetic biomaterial to be customized with selected bioactivity to acquire certain desired properties that can regulate cell–material interactions [33].

Several modular ELRs produced as biomaterials have been designed to mimic the ECM properties of elasticity, cell adhesion, cell signaling (cell-responsive) and biodegradability. The first bioactive peptides inserted into the polymer chain were the well-known, general purpose cell-adhesion peptides Arg-Gly-Asp (RGD) [34], which is found in several ECM proteins [35–38], and Arg-Glu-Asp-Val (REDV) [39,40], which is included in the CS5 domain of fibronectin and is specific for endothelial cell binding [41]. Both these bioactive peptides showed similar cell-adhesion properties to human fibronectin.

A further property, namely degradation responsiveness, which results in replacement of the initial artificial support upon reabsorption, thereby allowing the renovation and replacement of natural ECM by living tissue, has been introduced into the more advanced ELRs, and their derived tissue engineering scaffolds, for tissue repair [40,42]. This process requires that specific cells are able to lead normal enzymatic processes, as occurs in natural ECM remodeling, principally through matrix metalloproteinases (MMPs). These enzymes, which are only expressed and secreted into the ECM when it needs to be renewed, act on specific sequences that are only present in ECM proteins. It is also known that, once released, some fragments of these hydrolyzed ECM proteins, known as 'matrikines', show strong bioactivity, including the promotion of cell differentiation, spreading and regeneration [43].

A sophisticated ELR has been biosynthesized to generate temporary scaffolds that support neural regeneration, thereby helping to repair injuries to the CNS. This ELR was engineered to contain different bioactive domains for cell adhesion and cell-induced degradation of the artificial scaffold, namely RGD cell-adhesion sequences to enable neural attachment and sequences sensitive to cleavage by urokinase plasminogen activator (uPA), a protease secreted locally at the tips of growing neurites to enable highly localized and tuneable degradation. These ELRs were chemically cross-linked into highly swollen hydrogels with controllable mechanical properties. In addition, it was found that increasing the density of RGD peptides present in the protein substrates led to increased cell adhesion and more extensive neurite growth [42].

One of the ELRs bioproduced by our group is an 877 amino-acid complex polymer (REDV-ELP biopolymer) whose monomer unit contains four different functional domains in order to achieve a suitable balance between mechanical and bioactive responses. The polymer framework contains two elastomeric pentapeptides (VPGIG and VPGKG), which provide the desired mechanical properties, stimuli-responsive nature, biocompatibility and cross-linking domains, the CS5 domain from fibronectin, which contains the cell-selective adhesion REDV motif [40], and the human elastin hexapeptide VGVAPG, which is targeted by the elastases that rearrange and renew the ECM. 3D matrices with controlled pore sizes formed from chemically cross-linked REDV-ELR have been obtained for use in cell culture, and salt-leaching and gas-foaming techniques have been used to improve the homogeneity of the pore size, the mechanical properties and their interconnectivity. The 3D culture of HUVEC endothelial cells has shown the suitability of these scaffolds, with infiltration of these cells inside the porous network being essentially complete [44]. A study of the physical changes that occurred as a result of their temperature responsiveness showed that the collapse due to the phase transition above Tt decreased the mean pore size by approximately 30%. This technique is therefore a straightforward approach for the synthesis of advanced scaffolds with tunable biological and physical properties suitable for 3D cell culture (FIGURE 3). This REDV-ELR has also been used as a substrate to culture cells from the ocular surface. Corneal wound healing requires cell adhesion and proliferation, both of which are mediated by the binding of epithelial membrane-bound integrins to substrate ligands such as fibronectin. The corneal epithelial cell–ECM mimicking ELR interaction was investigated, and a significant improvement in the adhesion and proliferation of the conjunctival epithelial cell line on the REDV–ELR-coated surfaces was observed. This enhancement could be due to the epithelial integrins and REDV peptides binding to the ELR substrate. The REDV–ELR-coated surfaces also supported the normal phenotype and functions of epithelial cells *in vitro*, thus confirming that this recombinant polymer resembling the ocular surface ECM is a suitable substrate for sustaining epithelial cell attachment and growth. This type of polymer may therefore be suitable for tissue engineering to restore vision by reconstructing the ocular surface. One of the potential applications we envision for this kind of protein-based polymer is the preparation of scaffolds to be used for ocular surface tissue engineering [45].

Hybrid scaffolds composed of collagen and increasing proportions of the REDV–ELR described above have been produced to mimic the natural collagen and elastin networks that contribute to a highly specialized biomechanical response in numerous tissues [22]. These hybrid hydrogels were obtained by enzymatic transglutaminase cross-linking, thus mimicking the natural cross-linking of structural proteins in the generation of functional tissue *in vivo*. REDV–ELR provides elastic elements in the collagen-based scaffolds and also increases their functionality, thus contributing to enhanced proteolytic sensitivity and endothelial cell adhesion. Cross-linking was found to affect the physicochemical properties of the ELR–collagen scaffolds, especially their porosity, temperature responsiveness and mechanical strength. The viability of different cell lines on these enzymatic cross-linked ELR collagen hydrogels was tested *in vitro*. The results of this study showed that increasing the proportion of ELR in the hybrid scaffold resulted in fibroblast antifouling, whereas endothelial cells displayed normal behavior and proliferation in the hybrid hydrogels. This specific colonization of the scaffolds by a specific cell type makes these scaffolds an attractive platform for biomedical applications, as varying the proportion of both materials should allow the optimal requirements for future applications, such as vascular-tissue or skin-wound healing, to be designed. In recent times, complex hybrid scaffolds containing an ELR and collagen have been produced with the aim of creating an innovative acellular arterial substitute. Thus, Caves *et al.* described the creation of artificial multilamellar

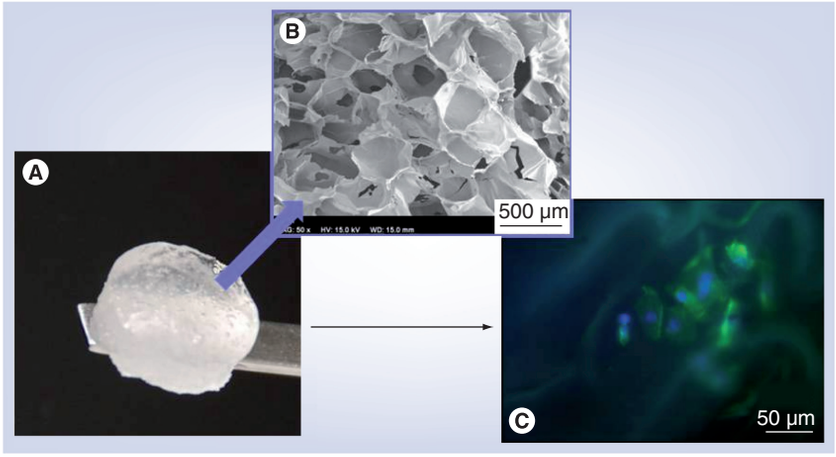

Figure 3. Macroporous thermosensitive hydrogels. (A) Macroscopic picture of swollen hydrogel. **(B)** Cross-sectional scanning electron micrograph. **(C)** Fluorescence microscopy with Phalloidin Alexa Fluor488 and 4′,6-diamidino-2-phenylindole staining.
Reproduced with permission from [44].

vessel-like scaffolds consisting of several layers of an ELR matrix with the monomeric sequence $([VPGAG]_2VPGEG[VPGAG]_2)_n$, which supplies appropriate mechanical properties, biocompatibility and cross-linking domains, reinforced with synthetic collagen microfibers [46]. The artificial vessels themselves were produced by embedding sheets of collagen microfibers in ELR solution, then cross-linking and rolling the resulting material. The authors analyzed the orientation and density of the two components to obtain the desired mechanical properties, including burst pressure, compliance, and suture retention strength.

Another type of hybrid scaffold has been synthesized for use as a provisional bone tissue scaffold. Thus, ELRs were amalgamated with chitosan to develop hydrogels with applications in bone tissue engineering, which requires temporary scaffolds to regenerate bone and improve its healing rate. These hydrogels can be implanted using a minimally invasive procedure by injection of a liquid that gels *in situ*. Chemically cross-linked chitosan hydrogels were prepared with an ELR coating containing an osteoconductive sequence on the preformed hydrogel. Incorporation of the ELR into the chitosan scaffold drastically improved the mechanical properties of this gel under physiological conditions and induced precipitation of calcium phosphate when the materials were soaked in simulated body fluid for seven days [47].

Ozturk *et al.* have demonstrated recently that RGD–ELR adsorbed on micropatterned poly(n-isopropylacrylamide) films is crucial to maintain the cell attachment in dynamic cell culturing. Thus, cell behavior was studied upon application of mechanical stress generated by changing the temperature from 37 to 29°C to shrink and stretch poly(N-isopropylacrylamide) (PNIPAM) films under *in vitro* conditions. This is a promising strategy to obtain smart cell carriers for bone formation [48].

Fusion protein purification

One useful application of the thermal phase transition of ELRs in the last few years has resulted in an improvement in the recovery and purification efficiency of fusion proteins as an alternative to conventional chromatography methods. Although chromatography-based protein-purification methods yield proteins of high purity, they also have several drawbacks that limit their industrial scale-up, including their high cost, the need to remove the affinity tags, when present, from the purified protein, which can adversely affect the structure or activity of the protein and may pollute the final protein recovery with unwanted residues, the fact that the sample volume is limited by the physical size of the column, and the need to perform several concentration steps after protein elution [49]. The temperature-dependent, reversible aggregation of ELRs provides a novel means to avoid this cost-intensive affinity chromatography by one of several strategies, including direct ELR tagging by simple ELR-based protein purification or ELR-coaggregation and purification by ELR-mediated affinity capture. In the first case, cleavage of the ELR moiety from the fusion protein by treatment with specific proteases could raise cost issues for large-scale protein production and could also result in some of the problems mentioned above inherent to protein recovery using conventional chromatographic procedures. However, a combination of ELR and intein technology, which is designed to induce self-cleavage, eliminates the need for post-purification enzymatic or chemical cleavage of the ELRs. In the second case, namely ELR-mediated affinity capture, the ELR can bind the fusion protein specifically and reversibly, thereby eliminating the need for post-purification enzymatic or chemical cleavage and possibly allowing re-use of the ELR-capture molecule [4]. In this regard, Araújo *et al.* have reported the microbial production of a functionalized high molecular weight subtilisin for controlled enzymatic hydrolysis of wool surface whose activity was successfully restricted to the cuticle of wool, thereby allowing a significant reduction in pilling, weight loss and loss of tensile strength of the wool fibers [50].

Functionalized surfaces

Recent progress made in the fields of nanoscience and nanotechnology has opened up novel frontiers in surface functionalization and characterization, which is often essential for endowing advanced materials and devices with desirable features. ELRs are excellent candidates for the development of smart surfaces as their sequence, length and stereochemistry can be closely controlled using recombinant technologies. This allows, amongst others, a precise nanometric-scale control of the position where functionality is located along the polypeptide chain and leads to an extensive potential for the self-assembly and other advanced functionalities displayed by these systems [7].

Surfaces modified with stimuli-responsive ELRs have been produced by Chilkoti's group, who refer to this technique, where an ELR is covalently micropatterned onto a glass surface against an inert background, as the 'thermodynamically reversible addressing of proteins' (TRAP) [51,52]. This TRAP technology enables the reversible, spatio-temporal modulation of ligand-binding triggered by the phase transition of an ELR at the solid-liquid interface and can be applied in different systems for bioanalytical applications, such as protein-based microsensors for detecting single biomolecules. The adhesion of other binding proteins coupled to designed polypeptides onto solid surfaces by hydrophobic interactions has also been reported [53]. Another fusion protein combining the RGD sequence, EGF and a hydrophobic sequence into one molecule has been shown to have both cell-adhesive and growth-factor activity, whilst its hydrophobic sequence contributed to assembly of the RGD and retention of its activity on a solid-phase surface. This fusion protein could therefore prove to be of use for wound healing and tissue regeneration [54].

Another simple technique commonly used in the field of ELRs is the layer-by-layer deposition of ELR polyelectrolytes to generate bioactive surfaces to modulate cell response. Costa *et al.*, for example, have developed thermoresponsive thin coatings by electrostatic self-assembly (ESA) [55], which involves simple deposition of an ELR containing the cell-attachment sequence RGD dissolved in aqueous solution onto chitosan surfaces. The thermoresponsive behavior of these coatings has shown that these systems can be exploited for tunable cell adhesion and controlled protein adsorption by nanoscale surface tailoring.

Aqueous ELR solutions are able to form interesting nano-architectures, thus providing highly reproducible nano- and micro-structured surfaces as both '*in situ*' implantable platforms or as substrates with well-controlled microtopography, in a simple, one-step process by heating to slightly above the T_t. Selection of the appropriate sequence for the biomimetic modification of surfaces with ELRs has resulted, for example, in the production of biometallic implants with osteostimulative properties by polymeric deposition with an optimal combination of random surface topography and bioactivity [56]. A significant aspect of the biological control over material formation in these biomineralization processes involves protein/inorganic interactions [1].

Fibers

The use of polymer nanofibers for biomedical and nanotechnological applications has many advantages. Recent uses of these materials include tissue engineering, medical implants, biosensors and drug release, amongst others [57]. The most popular and widely used technique is known as electrospinning, whereby a high voltage is applied to generate an electrically charged jet of polymer solution, which dries to leave a polymer nanofiber network containing fibers with diameters ranging from a few micrometers to less than a hundred nanometers. The first elastin-mimetic protein fibers were produced from a genetically engineered ELR [58]. Different morphological patterns, such as beaded fibers, thin filaments, or broad with a ribbon-like appearance, were subsequently obtained by varying the solution concentration. And as in other ELR constructions, the inclusion of specific biofunctionalities may provide a wide range of possibilities (FIGURE 4).

Recently, core-shell nanostructured cadmium selenide (CdSe) nanoparticles with a shell of ELRs have been used as building blocks to fabricate functional 1D nanostructures. The ELR stabilizes the CdSe nanoparticles in an aqueous medium and controls their nucleation, growth and spatial distribution, thus leading to the self-assembly of core-shell building blocks into fibril architectures. The use of nontoxic nanofibrous materials has shown that the combination of different materials with ELRs is a promising strategy to find new applications in fields such as medical nanotechnology and the diagnosis and treatment of various diseases [59].

Drug delivery

The combination of genetic engineering techniques as ELRs has permitted the incorporation of targeting peptides, such as cell-penetrating domains [60,61] or receptor ligands, and the

introduction of reactive sites for chemical conjugation drugs [62] or fluorescent probes [63] into the ELR sequences. The polymer self-assembly process of ELRs may also boost the formation of stable micro- and nanospheres that are able to trap active substances and can be used for subsequent drug-delivery applications [64,65]. The combined release of bone morphogenetic protein (BMP)-2 and-14 by exploiting the inverse temperature transition of poly(VPAVG) in a sustained way over a period of 14 days has been reported to show significant potential for future bone tissue engineering applications [65]. More recent studies showed a hysteresis behavior in this polymer, with thermal absorption/release of components depending on the salt concentration of the polymer solution [66]. Electrospraying, which is a versatile, efficient and flexible technique, has also been used to generate nanoscale, bioresponsive, peptide-based particles with defined morphology that can encapsulate drugs, such as the chemotherapy agent doxorubicin [67,68].

This ordered nanoscale assembly is sometimes driven by the incompatibility of the constituents of amphiphilic di- or tri-block polymers, which can give rise to the formation of monodisperse micelles in a narrow size range or fluid-filled sacs or vesicles [69]. ELR-based block copolymers can be tailor-made to form smart, self-assembling, protein-like micellar systems with controlled structure and function. For instance, Chilkoti *et al.* have focused on potential therapeutic applications by increasing the affinity of the ELR-targeting vehicle of heat-triggered reversible ELR-containing micelles [6,70]. Similarly, Dreher *et al.* have utilized diblock ELRs with an N-terminal peptide ligand to form multivalent spherical micelles when heated slightly above body temperature. The critical micelle temperature is controlled by the length of the hydrophobic block, and the size of the micelle is controlled by both the total ELR length and hydrophilic-to-hydrophobic ratio. This study also identified a subset of elastin-like block co-recombinamers that could be useful for drug targeting by thermally triggered multivalency [71]. Recently, Kim *et al.* have developed stabilized nanoparticles through the use of disulfide cross-linking between two amphiphilic diblock polypeptides modified with cysteine residues incorporated at the core–shell interface that could be stable under complex, physiological conditions [72]. Most of these studies involve diblocks, although there are also some examples based on triblocks, including one where a reversible change in micelle compacticity was triggered by a helix-to-sheet protein folding transition upon raising the temperature above T_t [73].

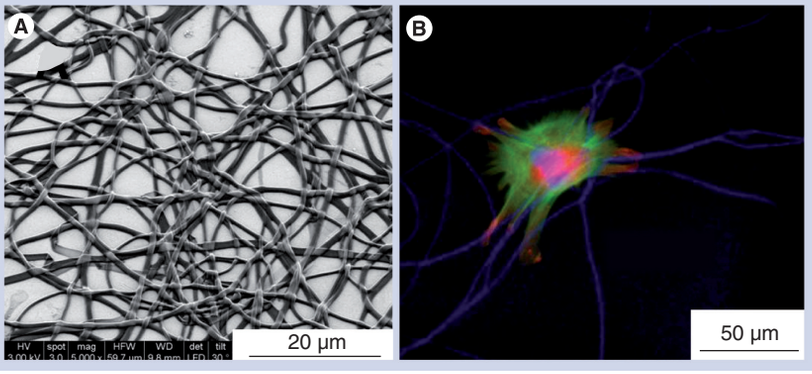

Figure 4. elastin-like recombinamer nanofibers. (A) Environmental scanning electron microscope micrograph of chemically crosslinked nanofibers obtained after random deposition of an Elastin-like recombinamer (ELR)–Arg-Gly-Asp (RGD) aqueous solution using the electrospinning technique. **(B)** Fluorescence microscopy image of a primary human foreskin fibroblast cell (HFF1) specifically adhered to ELR–RGD nanofibers. Vinculin staining of the focal adhesions shows a strong integrin-mediated cell–material interaction. Nucleus and fibers (blue), F-actin, (green), and vinculin (red).

Hybrid nanomaterials

Other biomedical applications for which the use of ELRs has been proposed are based on functional inorganic-ELR hybrid nanostructured biomaterials for advanced diagnostic and therapeutic applications. These hybrid materials may help to overcome issues related to the poor solubility of the inorganic component in aqueous environments, stability and toxicity, but have also been contemplated for the manufacture of specific biosensors and detectors and more efficient nanoprobes with multiple response properties and sensitivities, such as pH, temperature, light, concentration of ionic species and others, for drug- and gene-delivery applications in targeted therapy [74,75].

The 'one-pot' ELR-mediated synthesis of smart gold clusters has been reported recently by our group (Figure 5). This ELR promoted the formation of 2D linear arrangements of gold clusters with interparticle distances ranging from 10 to 40 nm. Furthermore, the gold-biopolymer hybrid displayed spectroscopic properties (UV-vis absorption) that could be modulated by varying the pH and temperature of the environment as a result of the reversible aggregation-expansion of gold particles [76].

In a subsequent step, Álvarez-Rodríguez *et al.* have explored the use of cyclodextrins and the formation of alkanethiol mixed-monolayers over the previously described hybrid ELR in order to decouple the photoisomerisation of azobenzene from the bulk phase absorption [77]. If they can be tuned to work under physiological conditions,

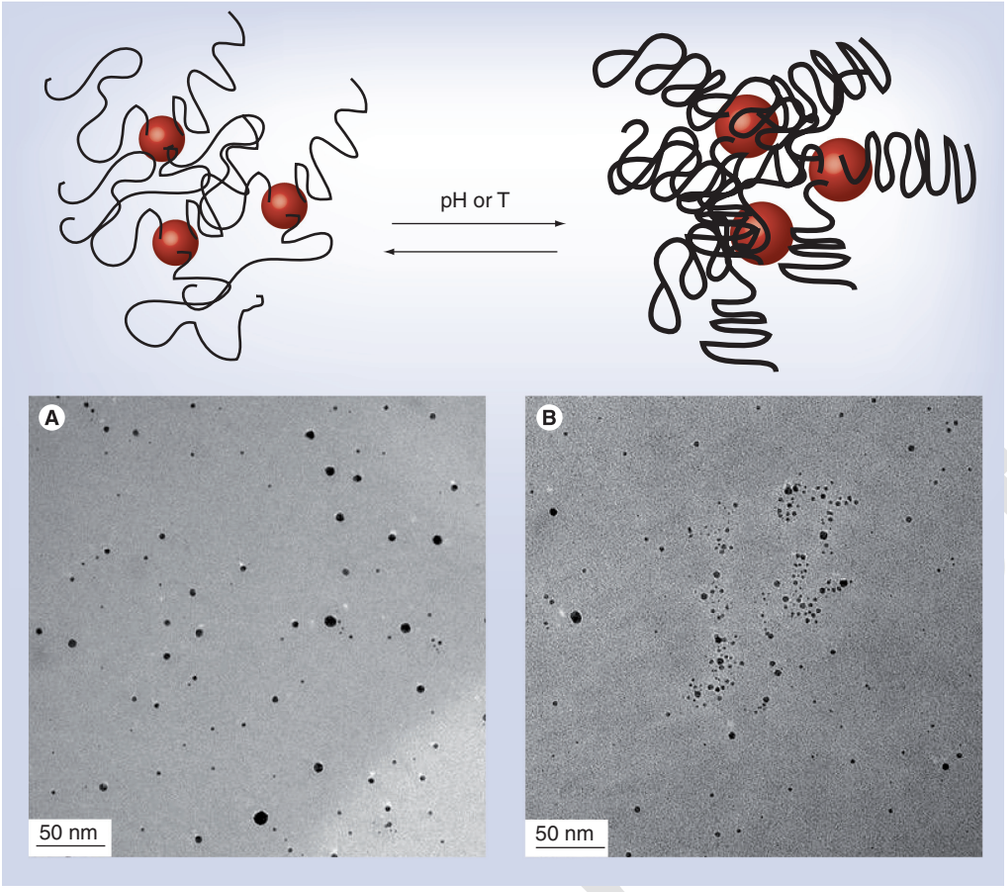

Figure 5. Self-assembly exhibited by a gold–elastin-like recombinamer hybrid as a function of pH and temperature. Cryo-transmission electron micrographs obtained at pH 4, at **(A)** 15°C and **(B)** 35°C.
Reproduced with permission from [74].

these smart gold–ELR hybrids could be interesting for the development of multistimuli biosensors and drug-delivery applications.

Conclusion & future perspective

Sequence selection during the construction of ELR-based supports with specific physicochemical and mechanical properties will always depend on their intended applications. The wide spectrum of biomedical and biotechnological applications in tissue engineering, protein purification, drug delivery and surface engineering are only some examples of the enormous potential of these versatile materials. In tissue engineering, the tailored inclusion of bioactive domains along with the possibility of tuning the physical properties by changes in the amino acid composition, inclusion of cross-linking or self-assembling domains amongst others to induce specific cell colonization makes these scaffolds an attractive platform for future applications, such as vascular-tissue or skin-wound healing.

The potential for ELRs to self-assemble in response to environmental changes makes them hugely attractive for the fabrication of promising nanodevices for numerous reasons, including the ability to load them with drugs during hydrophobic self-assembly above T_t under mild conditions of pH or in the absence of organic solvents, the possibility to target drug delivery to specific organs and tissues, and the ability to modulate subsequent drug release and optimize scaffold degradation by choosing an appropriate amino acid sequence for the polymer.

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Executive summary

- Elastin-like recombinamers (ELRs), obtained by repeating sequences found in natural elastin, are produced by genetic-engineering techniques with an extraordinary degree of complexity and control over their architecture and physicochemical properties.
- They are monodisperse, highly biocompatible, stimuli-responsive and can include diverse functionalities along the polypeptide chain.
- Their wide spectrum of biomedical and nanotechnological applications in tissue engineering, protein purification, drug delivery and surface engineering are only some examples of their enormous versatility.
- The tailored incorporation of bioactive domains, such as cell-binding peptides, together with the possibility of tuning the physical properties, which are retained in the ELR-derived substrates, makes them potentially useful materials for tissue engineering.
- Their self-assembly and stimuli-responsiveness properties are reversible at either a structural or a functional level. Furthermore, the increasing need for new systems with multiple response properties and sensitivities (pH- and light-sensitivity, drug- and gene-delivery, amongst others) that improve specificity and activity suggests the potential advantages of these materials in the field of nanodevices for targeted applications.

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