Application of elastin-mimetic recombinant proteins in chemotherapeutics delivery, cellular engineering, and regenerative medicine

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With the remarkable increase in the fields of biomedical engineering and regenerative medicine, biomaterial design has become an indispensable approach for developing the biocompatible carriers for drug or gene cargo and extracellular matrix (ECM) for cell survival, proliferation, and differentiation. Native ECM materials derived from animal tissues were believed to be the best choices for tissue engineering. However, possible pathogen contamination by cellular remnants from foreign animal tissues is an unavoidable issue that has limited the use of native ECM for human benefit. Some synthetic polymers have been used as alternative materials for manufacturing native ECM because of the biodegradability and ease of large-scale production of the polymers. However, the inherent polydispersity of the polymers causes batch-to-batch variation in polymer composition and possible cytotoxic interactions between chemical matrices and neighboring cells or tissues have not yet been fully resolved. Elastin-like proteins (ELPs) are genetically engineered biopolymers modeled after the naturally occurring tropoelastin and have emerged as promising materials for biomedical applications because they are biocompatible, non-immunogenic, and biodegradable, and their composition, mechanical stiffness, and even fate within the cell can be controlled at the gene level. This commentary highlights the recent progresses in the development of the ELP-based recombinant proteins that are being increasingly used for the delivery of chemotherapeutics and to provide a cell-friendly ECM environment.

Advantages of Recombinant ELP Engineering

ELPs belong to a family of recombinant proteins consist of the VPGXG pentapeptide, where the guest position X accepts any amino acid except for proline. One favorable feature of the ELPs is responsiveness to temperature change; solubilized ELPs associate with each other above a certain transition temperature and form self-assembled coacervates comprising β-spiral structures. The thermal sensitivity of ELP has been exploited in various biomedical engineering fields that benefit from coacervation at the target site where they form an aggregating drug repository or a solid matrix, thus providing a mechanism for sustained release of the co-localized drugs or acting as a mechanically robust scaffold. Currently, three DNA manipulation methods, iterative ligation, recursive directional ligation, and random concatemerization, are being used to oligomerize the monomer gene of repeating peptide into the gene encoding the protein of interest (Fig. 1). Implementation of such molecular biology techniques in ELP production is highly advantageous; the genetically encoded design of ELPs permits precise control over their molecular composition and chain length to meet the end users' applications. Therefore, through the recombinant ELP engineering, bioengineers can modulate the architectural complexity as well as the diverse...
localization to tumor site but also to overcome the drug resistance in the treatment of multidrug-resistant cancers by surpassing cellular efflux process. 4

The recombination of ELP gene with the genes for cell-penetrating domains, cytokines, or cell growth inhibitory peptides leads to a fusion form of ELPs to overcome susceptibility to degradation and poor tumor penetration in vivo (Fig. 2B). Noticeably, Raucher’s group engineered three genes of cell-penetrating Bac, ELP1, and helix 1 (H1) of c-Myc (cellular homolog of the v-myc avian

Drug and Gene Delivery
Positioning of amino acids such as cysteine, lysine, glutamic acid, and aspartic acids at the guest residues allows covalent cross-linking of the chemotherapeutics to the backbone of ELP molecules. Therefore, to enhance the accumulation of chemotherapeutics at the tumor region that is heated by local hyperthermia, thermally responsive ELPs as the drug carrier have ever been widely used in targeted drug delivery and controlled release of radionuclides, chemical drugs, and peptide therapeutics to solid tumor (Fig. 2A). In particular, the anticancer efficacy of ELP-doxorubicin4 and ELP-geldanamycin5 conjugates emphasizes that the ELP-drug conjugation is a useful mean not only to take advantage of thermally-induced localization to tumor site but also to overcome the drug resistance in the treatment of multidrug-resistant cancers by surpassing cellular efflux process. 4

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Figure 1. Schematics of iterative ligation (A), recursive directional ligation (B), and random concatenation (C).

Figure 2. Schematic drawings of multifunctional ELP (A), ELP micelle (B), and ELP polyplex (C).
myelocytomatosis viral oncogene) into a single DNA sequence.13 The fusion form of ELP, Bac-ELP1-H1, thus produced significantly reduced tumor growth in an orthotopic mouse model of breast cancer by blocking the activity of the oncoprotein C-Myc. In a similar way, Setton’s group synthesized a recombinant protein of ELP-tTNFR1I by fusion of the genes for ELP and the 3-dimeric fusion of RGD-containing ELPα (TNFox) receptor II (tTNFR-II). In vitro bioactivity using murine L929 fibrosarcoma cells, the fusion protein shows antagonistic effects on TNFox-mediated cytotoxicity. The results indicate that the fusion protein of ELP and tTNFR1I retains functionality of both domains, supporting the potential use as an immuno-modulator therapeutic. Chilkoti’s8 and MacKay’s9 groups, respectively, fused the CD13 ligand NGR tripeptide and Knob domain of adenovirus serotype 5 fiber protein to the N-terminal of diblock copolymers composed of hydrophilic block and hydrophobic domains. The resultant proteins, NGR-ELP[V1A8G7][A][ELP][V10] and Knob-ELP[V1A8G7][V1PVG][G]V self-assemble into monodisperse spherical micelles presenting NGR or Knob sequence on their coronas. The NGR-ELP[V1A8G7][A] and ELP[V10] selectively localizes to the tumor vasculature with an increased vascular retention and extravascular accumulation and the Knob-S4H8H shows more intracellular vesicular uptake into lysosomal compartments. As such, the multivalent presentation of specific ligands by micelle self-assembly on the ELP nanoparticles is a potentially useful strategy to develop new class of drug-loaded architectures that target a unique uptake mechanism.

When positively-charged ELPs interact with negatively-charged molecules such as plasmid DNA, they condense into nano-sized polyplexes.14 Therefore, cationic ELPs act as a promising carrier for DNA therapeutics delivery through ionic complexation with plasmids or siRNA. In analogy with fusion forms of ELPs, combinatorial use of fusogenic peptides with the cationic ELPs would improve the siRNA-mediated silencing of oncogenes by enhancing endosomal escape of polyplexes (Fig. 2C).
RGD-mediated signaling cascades, which eventually upregulate the expression of CAMs and ECM proteins or promote F-actin assembly. However, signaling molecules that are activated upon REP-cell interactions remain to be identified.

To assemble multilayered films or structures, researchers have exploited the electrostatic attraction between opposite charges as the driving force for layer-by-layer (LbL) deposition. Rajagopalan’s group constructed nano-scale multilayer (LbL) deposition. Rajagopalan’s charges as the driving force for layer-by-layer electrostatic attraction between opposite structures, researchers have exploited the interactions to remain to be identified.

Gevhre’s group fabricated biomimetic LbL multilayer in controlling cellular functions. Herrmann’s group fabricated LbL coatings prepared via isothermal adsorption at 4 °C. The reactivity of γ-amino groups toward cysteine in the repeating pentapeptide facilitates hydrogelation through the formation of H2O2-mediated disulphide bonds. Substituting the guest amino acid with cysteine in the repeating pentapeptide facilitates hydrogelation through the formation of H2O2-mediated disulphide bonds.

**Figure 3.** Differentiation of neuronal cells on the REP coatings prepared via isothermal adsorption at 4 °C (A) or thermally induced coacervation at 37 °C (B and C). (A) and (B) show fluorescent images of the expression of neuronal class II β-tubulin in N2a neuroblastoma cells (green color) and neural progenitor cells (red color). Blue color corresponds to nucleus. (C) is the phase contrast microscopic picture of neurite extension from human SH-SY5Y neuroepithelial cells. Cells were cultured in the absence of ELP and presence of ELP and ELP50.

**Hydrogel for Regenerative Medicine**

Given the fact that mammalian cells exist in 3D environments in native tissues, it is understandable that 3D matrix systems, regardless of their biomaterial identity, have consistently been found to enhance cell growth and differentiation. With the dimensionality of native tissue, ELP-based hydrogels could serve as models for understanding the effects of matrix stiffness on cell functions (Fig. 4). Several lysine-containing ELP block copolymers have been produced by systemically incorporating lysine residues into the guest position of elastin modules. The reactivity of γ-amino groups toward chemical linkers such as glutaraldehyde, phosphonium compounds, genipin, and tri-succinimidyl aminotriacetate leads to the formation of interconnected hydrogel networks. Chemical derivatization of γ-amino groups into cross-linkable functionalities to formulate hydrazine-linked hydrogels has been achieved. Transglutaminase-catalyzed cross-linking allows cell encapsulation to occur under mild reaction conditions. Substituting the guest amino acid with cysteine in the repeating pentapeptide facilitates hydrogelation through the formation of H2O2-mediated disulphide bonds. Hydrogels of ELPs exhibit typical characteristics of covalently cross-linked networks as the elastic module is larger than the viscous module and is independent of the frequency in dynamic oscillatory sweep tests. The swelling and mechanical behaviors of chemically cross-linked ELP hydrogels are dependent on protein concentration, molecular weight, and lysine or cysteine content of the monomeric sequence. Tailored ELP hydrogels with modular bioactive peptides and matrix stiffness regions have been utilized to understand the systemic or independent effects of each module on various types of cells, including dorsal root ganglia, human umbilical vein endothelial cells (HUVECs), and embryonic or adult stem cells. Considering the successful carriage matrix synthesis, cardiomyocyte development, and increased HUVEC
proliferation, this author expects that hydrogel engineering would be more extensively used to formulate 3D micro-environments for musculoskeletal and vascular tissues.

Conclusions and Future Prospects

Over the past 30 y, many cell culture experiments have included first-generation ELPs that are composed only of VPXGG repeats or functionalized with cell penetration sequences, apoptotic peptides, or simply with cell adhesive integrin ligands. The results of the tests clearly proved the potential of ELPs as smart biomaterials that are suitable for targeted drug delivery via locally-induced hyperthermia and modifying 2D and 3D targeted drug delivery via locally-induced smart biomaterials that are suitable for clear evidence of the efficacy of ELP matrix on the repair of the injured tissues within ex vivo or in vivo models is very low. As described above, the effects of ELP-based scaffolds have been tested mainly for the replacement of load-bearing tissues and organs such as cartilage and intervertebral disc and are being evaluated in vascular or cardiac tissue engineering. More biological investigations with animal models are required before using ELPs in clinical practice. Along with the efforts to recover the functionality of damaged tissue, creation of new bioactive ELPs will be continued to help resolve the critical obstacles in tissue repair. In particular, stem cell transplantation has emerged as an important cell-based therapy in regenerative medicine, specially, for the treatment of neurodegenerative diseases, and thus, the use of first-generation ELPs in neural engineering is expected to increase. Therefore, researchers would likely focus their attention on designing new fusion forms of ELPs that are tailored to have multifunctional modules similar to those found in signaling proteins or growth factors that are known to influence the survival and differentiation of neuronal stem and progenitor cells. In such cases, the next-generation ELPs can provide more versatile biocompatible candidates applicable to create permissive microenvironments for stem cell transplantation into the brain tissues.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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