

Engineering Elastin-like Polypeptide Hydrogels: from Design Strategies to Cellular and Biomedical Applications

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Abstract Elastin-like polypeptide (ELP) hydrogels are a class of biomaterials precisely encoded through genetic engineering. Owing to their excellent biocompatibility, precisely tunable thermoresponsive behavior, and favorable biodegradability, they hold great promise in the biomedical field. This review systematically summarizes the core design strategies of ELP hydrogels and their associated advances in cellular and biomedical applications. The discussion begins with an overview of the diverse physicochemical crosslinking strategies underlying the formation of ELP hydrogel networks. It then highlights the design of biomimetic extracellular matrices through the integration of functional domains. This approach promotes the application of ELP hydrogels in 3D cell culture and tissue engineering, such as cartilage and vascular regeneration. The review further examines applied research in cutting-edge fields, such as drug delivery, elucidating how the integration of material properties with biological functions can provide innovative solutions for complex tissue repair and regenerative medicine. Finally, this review offers a systematic theoretical reference and design rationale by discussing the future development of integrated ELP-based biomaterial platforms with dynamic, intelligent responsiveness and multifunctional domain capabilities.

Keywords Elastin-like polypeptide (ELP) hydrogel; Crosslinking strategies; Cell culture; Tissue engineering; Regenerative medicine

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1 INTRODUCTION

Elastin is a critical structural protein in the extracellular matrix (ECM). It is formed through the assembly and maturation of tropoelastin monomers into a highly crosslinked polymeric architecture.^[1,2] As the core component of elastic fibers, this archi-

ture provides excellent elasticity, resilience, and reversible deformation to mechanically dynamic tissues such as blood vessels, lungs, and skin. It enables tissues to withstand repeated tensile strain while rapidly recovering their initial conformation, thereby maintaining their structural integrity and functionality.^[3,4]

Inspired by the outstanding properties of natural elastin, researchers have sought synthetic or recombinant alternatives capable of replicating its functions in a controllable manner. In this context, elastin-like polypeptide (ELP) has emerged as a unique class of genetically engineered protein polymers that meet this need. Its fundamental structural unit typically consists of the repetitive pentapeptide sequence Val-Pro-Gly-Xaa-Gly (VPGXG), where the guest residue Xaa can be any amino acid except proline. This modular sequence architecture not only retains the key structural and thermodynamic properties of native elastin, particularly its inverse temperature transition behavior regulated by a lower critical solution temperature (LCST), but also confers upon ELP an unprecedented level of sequence programmability and tunable performance.^[5] Below the phase transition temperature (T_t), ELP chains are highly solvated and adopt a disordered conformation in aqueous solution. Upon heating above T_t , they undergo a reversible hydrophobic collapse and self-associate into aggregates,^[6] driven primarily by the dehy-

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dration of their nonpolar residues. At the molecular level, the physical origin of this sequence-programmable phase transition lies in the unique entropic elasticity mechanism of ELP. The elastic response arises from the synergistic contributions of conformational entropy, hydrophobic hydration entropy, and librational entropy of chain segments. Together, these synergistic contributions provide the physical groundwork for the subsequent design of macroscopic networks. Importantly, the T_t and the cooperativity of this phase transition can be precisely tuned through sequence-level parameters, including the chemical nature and distribution of the Xaa residues, the number of repeating units, and the overall molecular weight.^[7] Based on this thermally responsive behavior, ELP has enabled applications across multiple domains. At the biotechnology level, the reversible phase transition has been exploited to develop “inverse transition cycling (ITC)” purification, which allows efficient, low-cost, and chromatography-free purification of target proteins.^[8] At the biomaterials level, owing to its excellent biocompatibility, controllable self-assembly behavior, and ease of functionalization,^[9] ELP demonstrates broad application potential in cutting-edge fields such as smart biomaterials, controlled drug-delivery systems, and tissue-engineering scaffolds.^[10–18]

Building upon these molecular characteristics and programmable advantages, ELP offers a fundamentally new paradigm in hydrogel design: its material behavior is encoded directly at the amino acid sequence level, thereby enabling precise and deterministic control over macroscopic properties. Unlike conventional synthetic hydrogel precursors, whose properties are predominantly governed by monomer chemistry, crosslinking density, or post-processing conditions, the design of ELP hinges on its programmable pentapeptide motif. The guest residue at the “X” position can be flexibly substituted. Non-canonical amino acids or functional groups can be incorporated site-specifically. The number of repeats, molecular weight, and distribution of functional sites can be precisely controlled *via* DNA recombination technology. Together, these capabilities enable the customization of ELP from a genetic sequence to material performance. This intrinsic coupling between sequence information and functional properties enables deterministic control over the hydrogel’s formation kinetics, network topology, and ultimate mechanical performance through molecular design. As a result, ELP hydrogels establish a unique bridge across the interdisciplinary realms of polymer physics, protein engineering, and biomaterials science, achieving seamless integration from molecular motifs to macroscopic functional materials, thereby demonstrating highly programmable and customizable smart-material characteristics.

Benefiting from this precise sequence-to-property correspondence, ELP hydrogels inherit the excellent biocompatibility and tunable degradation behavior of natural elastin. Moreover, their programmable mechanical properties further endow the material with significant advantages in terms of mechanical stability and versatility in application scenarios. In cell culture and tissue engineering, such hydrogels can accurately mimic the physicochemical microenvironment of the ECM, effectively supporting cell adhesion, proliferation, and functional expression. Furthermore, they can be used to con-

struct smart responsive biomaterials for various applications, including wound repair, tissue regeneration, and adhesion.^[19–21] In summary, ELP possesses programmable mechanical properties, excellent biocompatibility, and versatile functional integration capability. These features make it an ideal scaffold material for constructing high-performance hydrogels, demonstrating indispensable value in the fields of cellular research, tissue repair, and intelligent biomedical applications.

This review systematically examines the design strategies of engineered ELP hydrogels and their applications in cellular and biomedical contexts (Fig. 1). First, hydrogel network fabrication methods based on different crosslinking strategies are summarized. Next, representative recent studies are incorporated to analyze the use of such materials in regulating cellular behavior and achieving tissue engineering functionalization. Finally, future design approaches, application directions, and research priorities are outlined.

2 DESIGN STRATEGIES FOR CROSSLINKING METHODS OF ELP HYDROGELS

Hydrogels, as a class of advanced functional materials, have garnered extensive research and application in the biomedical field due to their inherent 3D porous network structure, high water content, and excellent biocompatibility.^[22,23] The construction of their network architectures relies on various controllable crosslinking strategies, which can be broadly categorized into physical crosslinking and covalent crosslinking. To systematically review the relevant research progress, this section will focus on elucidating the crosslinking strategies applicable to ELP hydrogels within the structural classification framework of physical crosslinking and covalent crosslinking.

2.1 ELP Entropic Elastic Mechanism: Librational Entropy and Hydrophobic Hydration Behavior

The thermally responsive self-assembly of ELP represents a programmable and predictable phase transition behavior, and its physical origin can be traced to the entropic elasticity mechanism. At low temperatures, the ELP chains are generally in a disordered, soluble state. As the temperature rises above T_t , the ELP chains undergo hydrophobic folding and assembly, forming elastic aggregates. Experimental results indicate that this elasticity arises from entropic contributions (Fig. 2a). Therefore, based on studies of poly(VPGVG), it is possible to explain the elastic response of ELP by the librational entropy mechanism. During stretching, the damping of internal chain segment dynamics leads to a decrease in entropy.^[24]

From the perspective of solvent effects, water molecules also play a key role in regulating the entropic elasticity of ELP. Molecular dynamics simulations have demonstrated that when ELP is at physiological temperature and under moderate stretching, the orientational entropy of water molecules around hydrophobic groups decreases during stretching and recovers after the external force is removed. This reversible process indicates that hydrophobic hydration behavior also constitutes a significant source of the entropic elasticity in ELP (Fig. 2b).^[25] By measuring the force required to stretch a single ELP molecule in aqueous solution, single-molecule force spectroscopy further confirmed that when the elasticity

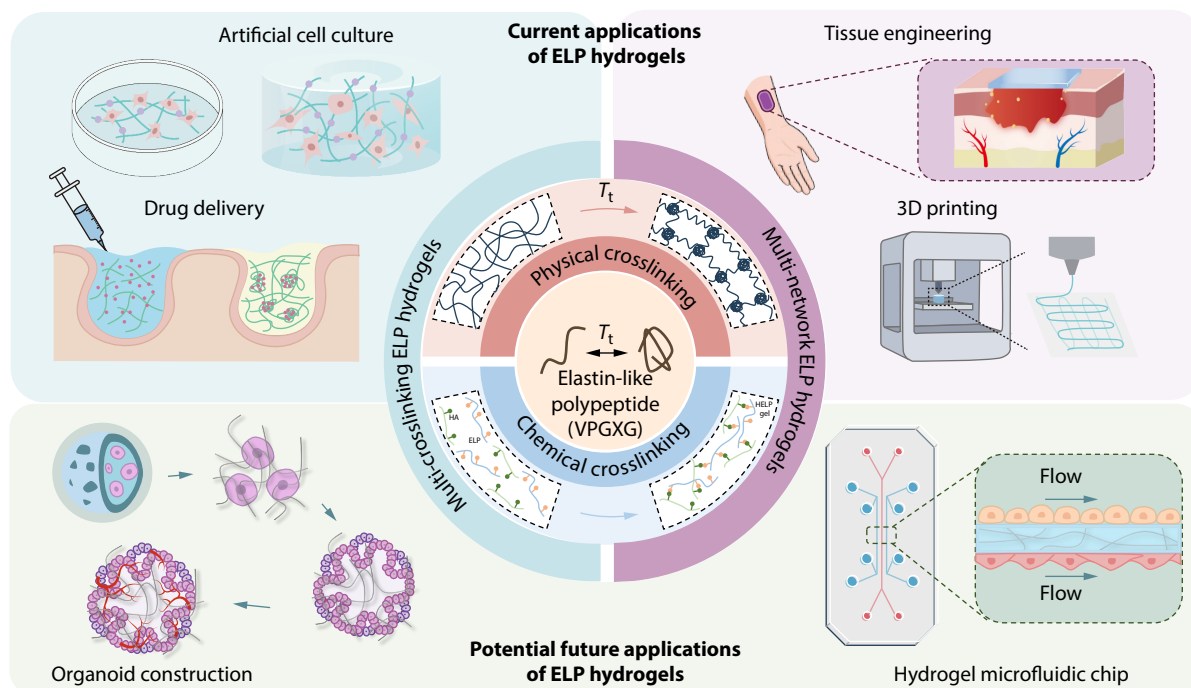


Fig. 1 Overview of ELP hydrogel design strategies and biomedical applications. This schematic illustration shows ELP hydrogel thermoresponsive behavior, sequence programmability, and diverse crosslinking strategies, enabling tunable mechanical properties and dynamic responsiveness. The illustration also shows representative applications in cell culture and tissue engineering, as well as future potential directions including 3D printing, organoid culture, and microfluidic systems.

of ELP molecules is modulated, the stretching force mainly depends on two components: the entropic elastic restoring force induced by stretching of the polypeptide backbone, and the force generated by changes in solvent-polypeptide interactions (Fig. 2c).^[26]

Molecular dynamics simulations and single-molecule force spectroscopy experiments have jointly confirmed that the fundamental reason for the excellent elasticity of ELP in the hydrated state lies in the cooperative entropic interplay between the polypeptide chain and water molecules. Upon dehydration, interchain hydrophobic interactions dominate, and the material may transition to a brittle state, thereby underscoring the critical role of water molecules in modulating its entropic elastic behavior.

2.2 Thermoresponsive Self-assembly as a Sequence-programmed Physical Network

Based on the aforementioned entropic elasticity nature, macroscopic ELP hydrogels can be designed using the inherent thermoresponsive behavior of ELP. This behavior is driven by the inverse thermal transition of the repeated VPGXG basic sequence and has precise sequence programmability. Specifically, when the ambient temperature exceeds the characteristic T_t , ELP chains undergo dehydration-driven hydrophobic collapse, which promotes interchain association via hydrophobic interactions and leads to the spontaneous self-assembly into physical crosslinking junctions. This process enables the direct construction of a thermally induced, physically crosslinked hydrogel based on a single-network architecture without the need for external crosslinkers or chemical reactions, thereby establishing thermoresponsive self-assembly as a sequence-encoded mechanism for physical network formation.

Early studies demonstrated that recombinant ELP with precisely controlled molecular weights designed *via* recombinant DNA technology could undergo *in situ* gelation at physiological temperature without the need for exogenous crosslinkers. For example, a single-component recombinant ELP with a molecular weight of approximately 36 kDa was sequence-regulated to set its T_t around 35 °C. When applied in cell culture at 37 °C, the ELP solution spontaneously formed physical crosslinks *via* hydrophobic aggregation upon exceeding T_t , thereby forming a 3D single-network scaffold that supports cell growth and differentiation. This system clearly illustrates how precise sequence control over hydrophobicity and chain length can directly regulate thermoresponsive gelation behavior.^[27]

Beyond simple lower critical solution temperature-driven aggregation, subsequent research has demonstrated that by systematically varying the proportion of hydrophobic guest residues (such as isoleucine and valine) and the number of repeat units, ELP solutions can undergo a unique form of hindered liquid-liquid phase separation when the temperature exceeds the transition point. This leads to the direct self-assembly of a homogeneous solution into a single-network, physically crosslinked hydrogel composed of nanoscale condensed peptide domains, which exhibits both enhanced mechanical properties and fully preserved thermoresponsiveness (Fig. 3a).^[28] This hindered liquid-liquid phase separation mechanism represents an important extension of the classical low critical solution temperature behavior.

While single-component ELP hydrogels are thermoresponsive, their functionality can be substantially expanded by incorporating additional peptide domains or synthetic polymer

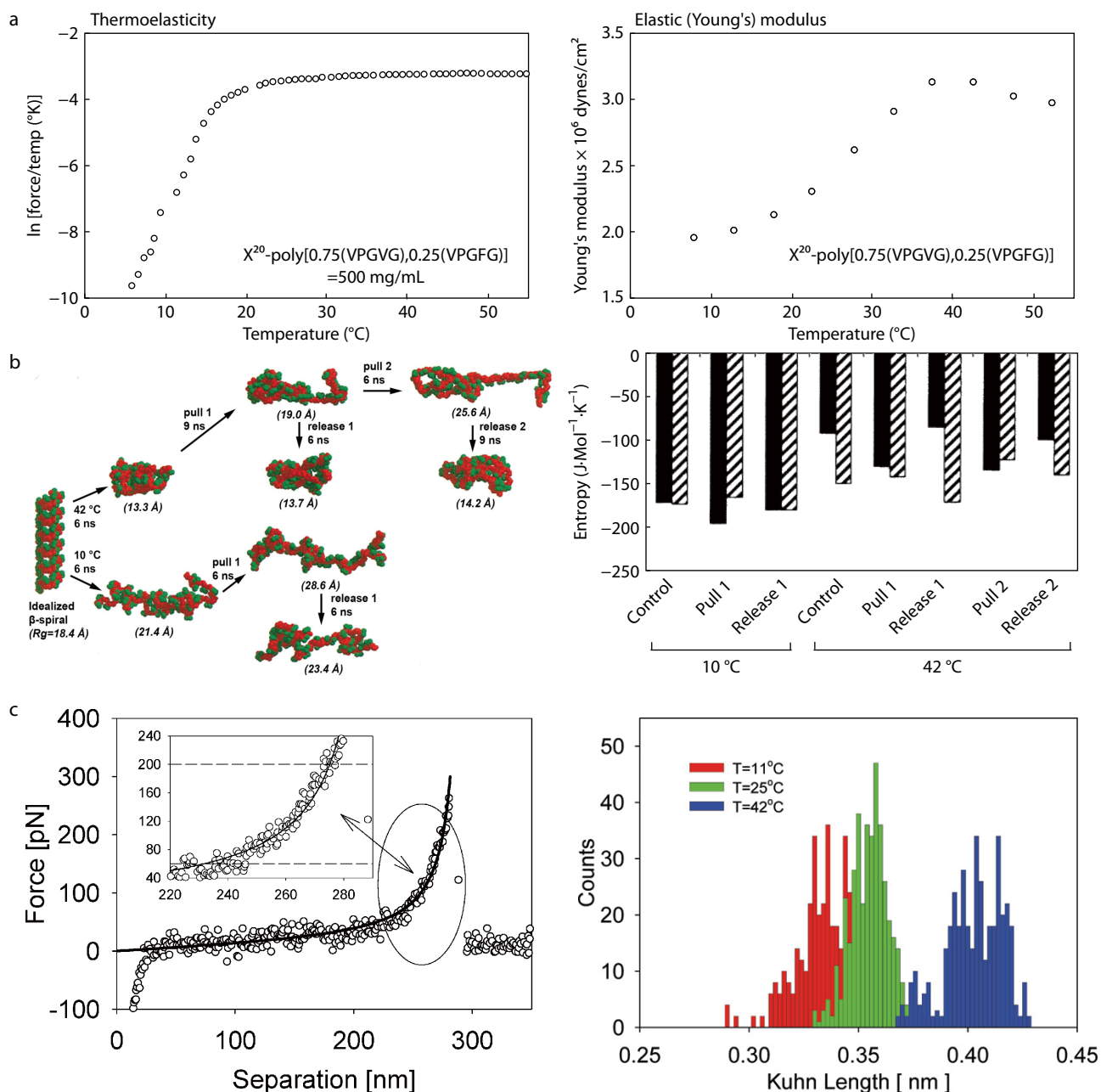


Fig. 2 Entropic elasticity mechanism underlying ELP thermoresponsiveness. (a) The slope of the $\ln(f/T)$ - T curve approaches zero (left), and the elastic modulus increases with rising temperature (right), demonstrating that the elastic force is entropically dominated and providing a thermodynamic basis for the librational entropy mechanism (Reproduced with permission from Ref. [24]; Copyright (1995), Ciba Foundation); (b) Conformational reversibility of the (VPGVG)₁₈ peptide during pulling or releasing cycles (left) and the changes in the orientational entropy of water molecules around its hydrophobic side chains (right) together indicate a hydrophobic hydration-driven entropic elasticity mechanism (Reproduced with permission from Ref. [25]; Copyright (2001), American Chemical Society); (c) Typical force-extension curve and freely jointed chain (FJC) model fit (60–200 pN) (left), and the increase of effective Kuhn segment length with temperature (from 11 $^\circ\text{C}$ to 42 $^\circ\text{C}$) (right) (Reproduced with permission from Ref. [26]; Copyright (2008), American Chemical Society).

blocks. Extensive research has further focused on integrating ELP with additional functional components to design multi-component hydrogel systems with enhanced properties. By genetically encoding ELP blocks in combination with other peptide domains, such as charged segments or coiled-coil motifs, researchers have directly expressed and formed copolymers. A representative design is the physically crosslinked single-network protein hydrogel (CUBE), which in-

corporates thermally responsive (AVGVP)₄₂-ELP blocks, length-tunable poly(aspartic acid) (polyD), tetrameric helical crosslinking peptides (CL), and cell-adhesive peptides (bRGD). This system achieves a solution-to-gel transition: the copolymer remains injectable at low temperatures, while heating to 37 $^\circ\text{C}$ triggers the formation of a transparent physically crosslinked hydrogel network.^[29]

In addition to sequence design, the regulation of sec-

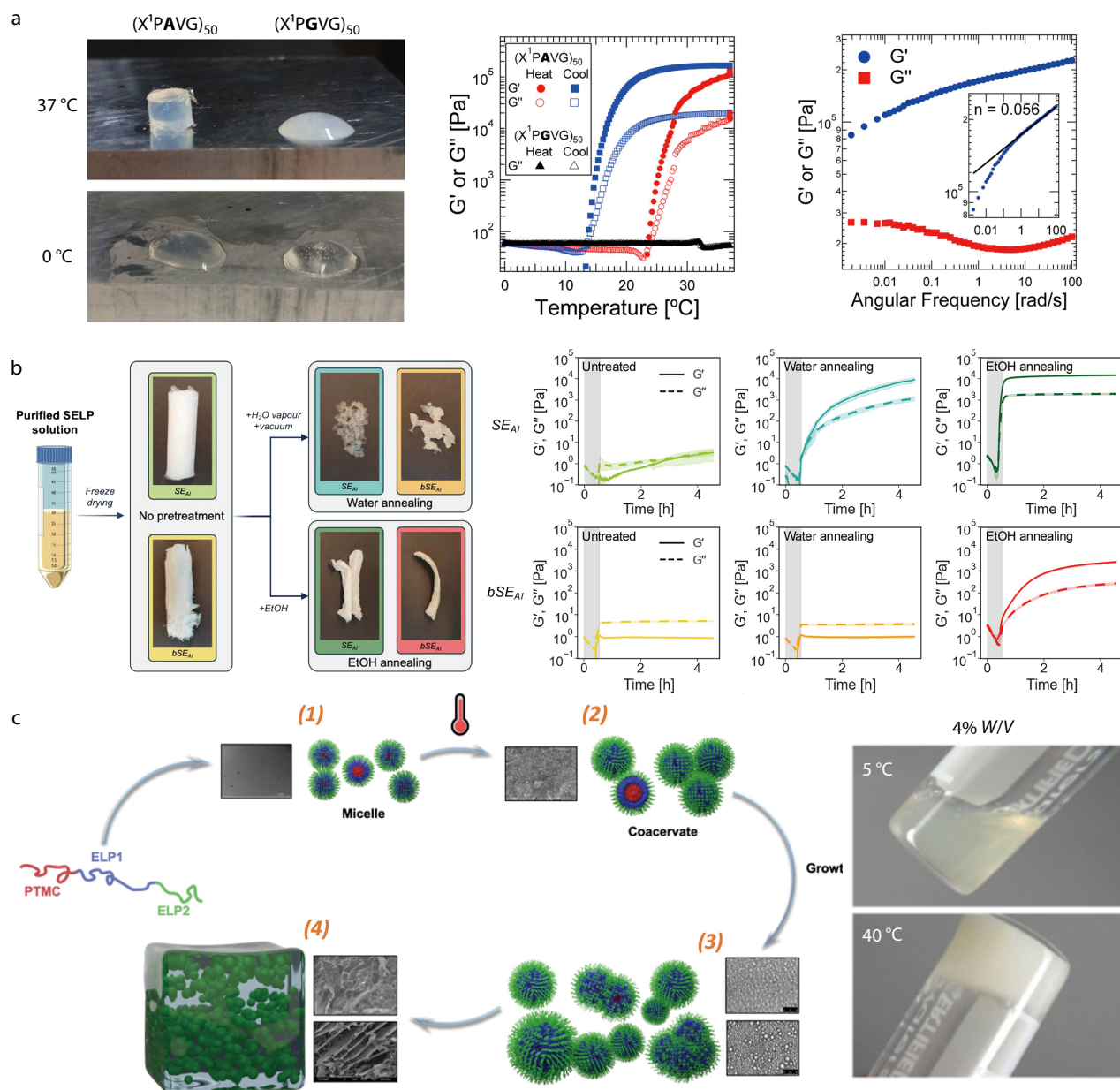


Fig. 3 Thermoresponsive self-assembly of ELP hydrogels. (a) Comparison of gelation behavior of thermoresponsive ELP solutions (Reproduced with permission from Ref. [28]; Copyright (2015), American Chemical Society); (b) Macroscopic images of dry SELPs after different treatments (left) and corresponding rheological characterization (right) (storage modulus G' and loss modulus G'') (Reproduced with permission from Ref. [30]; Copyright (2024), The Authors); (c) Schematic of the microstructural evolution of the ABC triblock copolymer (left) and its thermosensitive gelation behavior showing the formation of a free-standing hydrogel at 4% W/V upon heating from 5 °C to 40 °C (right) (Reproduced with permission from Ref. [31]. Copyright (2020), American Chemical Society).

ondary structures can further reinforce physically crosslinked ELP hydrogel networks. Taking silk-elastin-like polypeptides (SELPs) as an example, post-processing methods such as water annealing or ethanol annealing can significantly increase the β -sheet content without altering the primary amino acid sequence, thereby enhancing intermolecular β -sheet-mediated physical crosslinking and markedly improving the mechanical strength and structural stability of the hydrogel in aqueous environments (Fig. 3b).^[30] This strategy highlights that modulating the order-disorder balance of polymer chains serves as a key pathway for tailoring the mechanical

properties of ELP hydrogels, while also demonstrating that the ordering of higher-level structures is a critical factor in enhancing the stability of physically crosslinked networks. The underlying mechanism extends beyond the classical phase-transition process that relies solely on hydrophobic collapse.

Another example involves combining hydrophobic poly(trimethylene carbonate) (PTMC) blocks with thermoresponsive ELP blocks to construct an ABC triblock hybrid copolymer. In aqueous solution, this material self-assembles into nanomicelles with a PTMC core and an ELP corona. Upon heating above the T_g , the ELP blocks undergo reversible hy-

drophobic folding and aggregation. These, along with the hydrophobic PTMC microdomains, act as physical crosslinking points. This drives further fusion and interconnection of the micelles, leading to the formation of a macroscopic hydrogel with a micron-scale porous architecture, thereby achieving a rapid sol-gel transition governed solely by temperature (Fig. 3c).^[31]

In the research of ELP hydrogels, computational simulations and theoretical modeling have become indispensable tools for elucidating the mechanisms underlying their thermoresponsive self-assembly at the molecular level. Through all-atom molecular dynamics simulations, it has been systematically demonstrated that the phase transition behavior of ELP is a collective phenomenon. This phenomenon involves gradual conformational changes in individual chains coupled with an abrupt alteration in the properties of the surrounding hydration water. Specifically, heating triggers progressive chain folding, exposure of hydrophobic groups, and disruption of the hydration network, ultimately leading to inter-chain physical crosslinking.^[32] Furthermore, by establishing a single equation composed of three parameters, the T_t of ELP with a fixed composition has been quantitatively predicted as a function of chain length (L) and concentration (C). The model reveals that T_t is particularly sensitive to changes in both L and C within the lower concentration and shorter chain length regimes. This relationship provides a crucial theoretical scale for setting system dimensions and concentration conditions in simulations such as molecular dynamics (MD), as well as for interpreting the driving mechanisms of phase transition, including hydrophobic dehydration and chain conformational changes.^[33]

Collectively, these studies demonstrate that the thermoresponsive self-assembly in ELP hydrogels is not a generic physical phenomenon, but rather a sequence-programmed and predictable process, in which amino-acid composition, chain architecture, and higher-order structure jointly determine the formation, length scale, and mechanical response of the gel network.

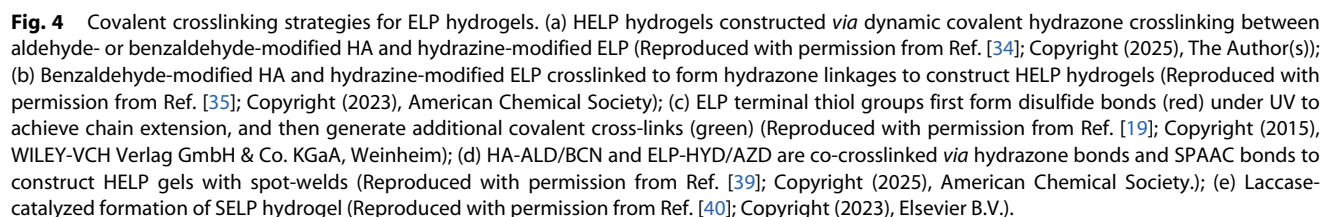
2.3 Covalent Crosslinking as an Extension of Sequence Design

The mechanical properties of ELP hydrogels are determined not only by their molecular characteristics, such as sequence design and molecular weight, but are also significantly regulated by the chemical nature and spatial density of crosslinking sites within the network. While single-network structures based on physical crosslinking offer favorable injectability and reversibility, their mechanical strength and long-term stability are often limited by the dynamic nature of non-covalent interactions. To achieve tougher and more durable material performance, research strategies have increasingly focused on incorporating covalent crosslinks into ELP networks, thereby constructing chemically crosslinked single-network architectures. Importantly, within ELP systems, covalent crosslinking should not be regarded merely as a post-hoc reinforcement of physical networks, but rather as a programmable extension of sequence design. By precisely positioning reactive residues and functional motifs along the backbone, this approach enables rational control over crosslinking chemistry, network topology, and ultimately mechanical performance, thereby endowing the hydrogels with

higher mechanical stability and tunable rigidity. This encompasses several major categories.

The first category encompasses functional group modification-mediated crosslinking, which involves the introduction of specific chemical moieties onto the side chains of ELP to facilitate the establishment of chemical crosslinks with other components. Among various strategies, the dynamic covalent hydrazone bond crosslinking approach has garnered significant attention due to its reversibility. A representative example is the HELP hydrogel system, wherein aldehyde/benzaldehyde-modified hyaluronic acid (HA) and hydrazide-modified ELP undergo crosslinking *via* dynamic covalent hydrazone bonds, ultimately forming a viscoelastic network with tunable stress relaxation behavior (Fig. 4a). The paramount advantage of this crosslinking strategy lies in its chemical orthogonality and modular design principle: the amino acid sequence of the ELP can be precisely encoded, and the crosslinking reaction responsible for constructing the network does not interfere with the biological activity of ligands. This characteristic enables independent and precise modulation of mechanical properties and biochemical signaling within the hydrogel matrix.^[34] In addition, this cross-linking strategy of benzaldehyde-based HA and hydrazine-modified ELP has also been used to screen different ligand combinations, thereby systematically studying the effects of biochemical signals on cell behavior (Fig. 4b).^[35]

The second category involves residue-encoded crosslinking strategies within covalently crosslinked ELP hydrogels, which rely on the introduction of specific amino acids into the ELP sequence to serve as reactive sites. Crosslinking can be achieved directly *via* the amino acid residues present in the constructed ELP polymer. Early work demonstrated that lysine-rich ELP, when chemically crosslinked with the electrophilic crosslinker bis(sulfosuccinimidyl) suberate under conditions such as phosphate buffer, yield single-network hydrogels with tunable mechanical properties.^[36] In subsequent studies, the authors further extended the crosslinking conditions by using not only bis(sulfosuccinimidyl) suberate but also disuccinimidyl suberate, allowing crosslinking in either DMSO or phosphate buffer.^[37] Through these two works, they collectively constructed responsive hydrogels with a well-defined microstructure and tunable mechanical properties. Subsequent studies utilized *E. coli* to directly express and purify ELP containing periodic lysine residues, which were then rapidly crosslinked in aqueous solution using the highly biocompatible β -[tris(hydroxymethyl)phosphino]propionic acid (THPP), forming chemically crosslinked ELP hydrogels.^[38] Building on this, tris(hydroxymethyl)phosphine (THP) is used as a crosslinking agent to rapidly form a covalent network through Mannich-type condensation with the amine groups on ELP and poly(ethylene glycol) bis(amine) (PEG bis(amine)) chains. The incorporation of hydrophilic PEG reduces the light scattering caused by ELP hydrophobic aggregation, thereby significantly improving the optical transparency of the hydrogel material. Since the ELP component provides cell-adhesive ligands, this design preserves the ability of ELP hydrogels to independently modulate mechanical properties and ligand density while enhancing transparency, thereby offering a platform that combines optical clarity with functional tunabil-



ity for 3D cell studies.^[41] Thiol-containing ELP forms intermolecular disulfide bonds under oxidants such as H_2O_2 , thereby constructing a single-network, chemically crosslinked, reversible thermoresponsive ELP hydrogel.^[42] In photochemical systems, numerous studies have demonstrated gelation strategies that form chemical crosslinks *via* photo-crosslinking modification. For instance, an early study reported a visible light-induced solid-state crosslinking strategy, the key to which lies in the controlled chemical modification and structural design of the ELP polymer. Based on the recombinantly expressed biomimetic elastic protein polymer poly((VPGVG)₄(VPGKG))₃₉, the lysine residues embedded within the protein backbone were subjected to acrylate derivatization. The functionally modified polymer was subsequently processed *via* electrospinning at an appropriate temperature to generate nanofibers. Upon incorporation of a visible-light photoinitiator system, solid-phase photo-induced crosslinking was triggered, resulting in the formation of intermolecular chemical crosslinks and the construction of a protein-based biomimetic material with a well-defined covalent network. This strategy not only enabled spatiotemporal control over the crosslinking process but also provided an effective paradigm for the chemical modification and crosslinking design of ELP hydrogels with controllable architecture and tunable mechanical properties.^[43] In subsequent study, cysteine residues were introduced at both termini of the repetitive ELP sequence ((VPGVG)_n), endowing each ELP chain with reactive thiol groups at its ends. Upon UV irradiation (360–480 nm) in the presence of the photoinitiator Irgacure® 2959, chain extension was first achieved through disulfide bond (—S—S—) formation, followed by radical-mediated transfer that generated additional covalent crosslinks between ELP chains. This process resulted in the rapid formation of photocrosslinked single-network hydrogels with tunable mechanical properties (Fig. 4c). This system demonstrates how precise molecular design enables the integration of covalent crosslinking sites directly into the polypeptide sequence, offering a strategy for the fabrication of structurally well-defined, biocompatible, single-component protein hydrogels.^[19]

The third category involves click chemistry crosslinking. Within the construction strategies of covalently crosslinked ELP hydrogels, click chemistry has garnered considerable attention due to its high efficiency, bioorthogonality, and mild reaction conditions, thereby offering a distinct regulatory dimension for the formation of ELP networks. Among these approaches, strain-promoted azide-alkyne cycloaddition (SPAAC), as a representative click reaction, has been extensively employed to rapidly crosslink azide and cyclooctyne functionalized ELP under cytocompatible conditions, thereby enabling cell encapsulation with second-scale gelation kinetics. A previous study modified lysine residues within ELP with either azide or bicyclononyne (BCN) groups, achieving rapid crosslinking on a timescale of seconds and demonstrating the capacity to independently tune both the mechanical properties and bioadhesive signaling of the resulting hydrogels.^[44] To further expand the design freedom of ELP hydrogels and prevent lysine residues within bioactive sequences, such as lysine-containing functional peptides including IKVAV and QK, from serving as crosslinking sites, a subsequent study de-

veloped a tyrosine-selective modification strategy. This approach employs azide-bearing 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) reagents to efficiently introduce azide groups onto tyrosine residues, which subsequently undergo SPAAC crosslinking with BCN-functionalized ELP. While maintaining rapid gelation within seconds and cytocompatibility, this strategy achieves compatibility with lysine-containing bioactive sequences, thereby substantially enhancing the chemical flexibility and functional programmability of ELP hydrogels for the construction of biomimetic microenvironments.^[45] Additional research has modified ELP with hydrazine and azide groups, while HA was functionalized with aldehyde and bicyclononyne (BCN) moieties, enabling the simultaneous introduction of dynamic hydrazone bonds and sparse, static SPAAC click crosslinks (Fig. 4d). This approach yielded a hybrid dynamic-static single-network hydrogel, in which a limited number of irreversible static crosslinks were strategically interspersed as anchoring points throughout the reversible dynamic network. This design imparts ELP hydrogels with combined advantages including stability, viscoelasticity, stress relaxation, and injectability.^[39]

The fourth category is enzymatic crosslinking. As an alternative to traditional covalent crosslinking,^[46] this approach primarily employs biological enzymes, such as transglutaminases and peroxidases, to catalyze covalent bond formation between native amino acid residues. It thus offers milder reaction conditions and superior biocompatibility. For instance, two distinct ELP variants were genetically engineered to contain lysine (K) and glutamine (Q) residues, respectively. Upon mixing, these variants undergo a reversible phase transition to form coacervate aggregates. The subsequent introduction of tissue transglutaminase catalyzes covalent crosslinking between glutamine and lysine residues on adjacent ELP chains, thereby constructing a structurally stable 3D network. This strategy successfully achieves injectability and *in situ* crosslinking, while also significantly enhancing the shear modulus of the resulting hydrogel. These findings demonstrate the efficacy and tuning potential of enzymatic crosslinking in fabricating injectable, biocompatible single-network ELP hydrogels.^[47] Subsequent study also confirmed that ELP hydrogel based on K-Q enzymatic crosslinking is stable and biocompatible.^[48] Another example introduces tyrosine and lysine into the SELP sequence *via* genetic engineering. First, the SELP polymer is assembled using the SpyTag/SpyCatcher system. Then laccase catalyzes the tyrosine residues to form dityrosine covalent bonds, while lysine assists in the crosslinking process. This results in an SELP hydrogel with a low swelling ratio, a porous structure, and good mechanical properties (Fig. 4e).^[40]

Overall, chemically crosslinked ELP hydrogels exemplify the integration of covalent network construction within a sequence-programmable framework, rendering covalent crosslinking in ELP hydrogels a predictable and modular design element and thereby expanding the design paradigm for ELP hydrogel networks.

2.4 Integrated Multi-network/Multi-crosslinking ELP Hydrogels Enabled by Coupled Physical and Covalent Crosslinking

Hydrogels, owing to their high water content and good biocompatibility, are widely used in biomedical fields such as tis-

sue engineering scaffolds and artificial cartilage. However, their mechanical properties often fall short of meeting the requirements for load-bearing capacity and fracture resistance in practical applications.^[49] While conventional single-network hydrogels can achieve moderate improvements in mechanical strength through covalent crosslinking, they remain pervasively limited by insufficient strength, low toughness, and a high propensity for fracture and crack propagation. These inherent drawbacks have severely constrained their further application in various biomedical contexts.^[50] Leveraging the sequence programmability of ELP, diverse crosslinking interactions can be integrated into a single hydrogel framework, either as spatially interpenetrated multi-network architectures^[51,52] or as multi-crosslinking modalities along the same network backbone^[53,54] (Fig. 5a). These crosslinking methods can endow ELP hydrogels with higher mechanical performance and multifunctionality.

A representative line of research involves the construction of chemically-physically crosslinked double network hydrogels. By virtue of their thermoresponsive characteristics, ELP can intrinsically function as the secondary network within a double network architecture, supplying dynamic and reversible physical crosslinking. One illustrative example is the design of an injectable double network hydrogel comprising dynamically crosslinked chemistry and thermally responsive engineered proteins. Specifically, hydrazine-modified elastin-like polypeptides (ELP-HYD) and aldehyde-modified hyaluronic acid (HA-ALD) are first mixed at ambient temperature to generate dynamic covalent hydrazone bonds, constituting the primary network. Upon heating, the thermoresponsive behavior of ELP drives the formation of physical crosslinking, establishing the secondary network (Fig. 5b).

These two integrated networks collectively confer shear-thinning behavior, injectability, as well as post-injection self-healing and thermoresponsive capabilities, thereby substantially enhancing hydrogel stability and reducing degradation kinetics. This system serves as a promising scaffold for long-term cell culture and induced differentiation.^[52]

In contrast to multi-network structures that are spatially interpenetrated yet lack direct connections, the formation of multiple crosslinks from different moieties—including ELP—along the same network backbone can also impart distinct properties to the hydrogel. One illustrative example involves the construction of a system in which a chemically crosslinked framework serves as the base, with ELP moieties attached to establish dynamic physical crosslinking based on their thermoresponsive properties. In this material, a photochemically crosslinked network of methacrylated gelatin (MGel) acts as the covalent backbone, while methacrylated ELP (MELP) is introduced as a multifunctional crosslinker covalently anchored within the network. When the temperature rises above the T_i of ELP, the ELP segments undergo collapse and aggregation via hydrophobic interactions (Fig. 5c), forming additional reversible physical crosslinks within the network. This leads to hydrogel contraction and consequently a significant enhancement in mechanical strength. This strategy, which couples chemical anchoring with physical responsiveness within a single network, offers potential not only for smart drug release but also for the development of 3D cell culture models with dynamically tunable mechanical properties.^[53] In addition to this approach of covalently anchoring ELP within the network, ELP can also function as thermoresponsive side chains rather than as a primary network

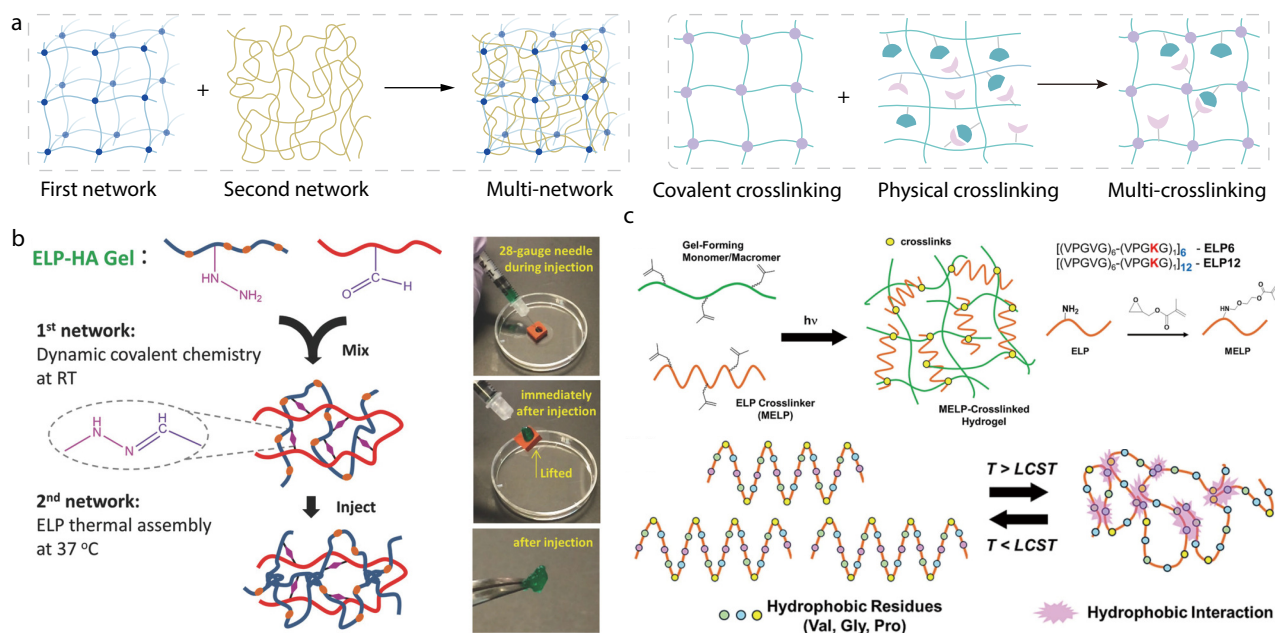


Fig. 5 Integrated multi-network / multi-crosslinking architectures for enhanced mechanical performance in ELP hydrogels. (a) Schematic diagram of multi-network/multi-crosslinked structure in ELP hydrogels (Reproduced with permission from Ref. [50]; Copyright (2024), Springer Nature Limited); (b) Schematic diagram of ELP-HA hydrogel formation (Reproduced with permission from Ref. [52]; Copyright (2017), WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim); (c) Schematic diagram of MELP-crosslinked hydrogel constructed through free radical copolymerization. MELP is anchored in the MGel chemical network as a crosslinker through free radical copolymerization (top), and ELP undergoes strong hydrophobic interactions above the T_i (bottom) (Reproduced with permission from Ref. [53]; Copyright (2025), Elsevier B.V.).

former. In these architectures, a chemically crosslinked backbone provides permanent structural support, while pendant ELP chains, rather than participating in covalent crosslinking, undergo reversible thermal collapse to introduce temperature-dependent physical entanglements. For example, one representative case involves the construction of a $V_n\text{-(GR)}_4$ block copolymer by genetically fusing ELP (VPGVG) $_n$ with the elastin-derived (GR) $_4$ sequence. Using a ruthenium-mediated photocovalent crosslinking method, the tyrosine-containing (GR) $_4$ units were covalently crosslinked to form the hydrogel network. The Tyr-free V_n -ELP blocks remained as uncrosslinked pendant side chains that aggregated above the T_v , thereby imparting tunable thermoresponsive mechanical properties to the protein-based composite hydrogel. This design demonstrates that ELP self-assembly can be decoupled from the primary network formation while still contributing dynamic physical reinforcement.^[54]

3 APPLICATION AREAS OF ELP-BASED HYDROGELS

As discussed above, the high water content of hydrogels provides an aqueous microenvironment resembling that of the ECM, while their porous architecture facilitates the exchange and diffusion of nutrients, metabolites, and signaling molecules. These characteristics render hydrogels indispensable in artificial cell culture and biomedical applications.^[55,56] Furthermore, ELP-based hydrogels, owing to their well-defined proteinaceous composition, exhibit superior biocompatibility. In this section, we highlight specific applications of ELP hydrogels in artificial cell culture and other biomedical fields.

3.1 Application of ELP-based Hydrogels in Artificial Cell Culture

The ECM-like microenvironment provided by ELP hydrogels supports the growth and proliferation of encapsulated cells. These hydrogels can also serve as advanced biomimetic scaffolds. They establish specific and functional interactions with diverse cell types, enabling in-depth investigation of critical biological processes such as cell migration, proliferation, and differentiation. To achieve such functions, a common design strategy is to introduce bioactive peptide sequences into the ELP hydrogel network (Fig. 6a). These bioactive peptides, typically derived from the functional domains of full-length native proteins, are characterized by shortened sequences and offer distinct advantages including high purity, prolonged functional activity, and enhanced environmental robustness (such as reduced sensitivity to pH and temperature fluctuations and lower susceptibility to proteolytic degradation), thereby facilitating more precise and targeted cell signaling.^[57,58]

Native ECM is a highly dynamic, hydrated network structure composed of water, structural proteins (including collagen, fibronectin, elastin, and laminin), proteoglycans, signaling molecules (such as growth factors and cytokines), as well as essential minerals and ions.^[59] Serving as a signaling hub for normal cellular physiological activities, the ECM plays an indispensable role in coordinating intercellular communication and precisely regulating cellular behavior.^[58] A key principle in the construction of functional biomimetic scaffolds lies in identifying bioactive short peptide sequences derived

from these ECM protein components that are capable of specific interactions with cognate cell surface receptors, thereby serving as fundamental building blocks for scaffold design.^[60] Here, we enumerate and describe several representative bioactive peptides that have been successfully integrated into ELP hydrogels during their fabrication as biomimetic scaffolds in Table 1.

When ELP hydrogels are engineered as biomimetic scaffolds for cell culture, it is necessary to consider the presence of sites capable of providing adhesive binding for cells within the hydrogel network. In most studies, the most extensively employed adhesive sequence is the RGD peptide. The RGD peptide specifically binds to integrin family receptors on the cell surface, thereby promoting stable adhesion of cells to the hydrogel matrix.^[61]

One representative example involves the design of a series of HELP-SPAAC hydrogels with tunable mechanical properties *via* a click reaction between ELP and HA. RGD peptide was incorporated as cell adhesion sites to facilitate cell anchoring and mechanosensing of the matrix. By culturing human mesenchymal stromal cells (hMSCs), human umbilical vein endothelial cells (HUVECs), and human induced pluripotent stem cell-derived neural progenitor cells (hNPCs) encapsulated within these hydrogels, the cells exhibited exceptionally high sensitivity and cell type-specific behaviors in response to matrix mechanical signals (Fig. 6b). These findings further demonstrate that precise and independent regulation of matrix mechanical properties is critical for directing cellular behaviors.^[62] One illustrative example is the previously described ELP hydrogel formed *via* bioorthogonal click chemistry, into which RGD peptide was incorporated prior to encapsulation and culture of hMSCs and mouse neural progenitor cells (mNPCs). The RGD peptide provided integrin-binding signals, thereby promoting cell adhesion and spreading. This study investigated cell viability, long-term culture, and phenotype maintenance (Fig. 6c), ultimately demonstrating that the engineered ELP hydrogel serves as an ideal platform for 3D stem cell culture.^[45] Furthermore, the study highlighted that future incorporation of additional bioactive peptides, such as the laminin-derived, lysine-containing IKVAV peptides, could endow the hydrogel with enhanced biological functionalities, including improved cell survival^[63] or facilitated neuronal differentiation and maturation.^[64]

Additionally, another study incorporated an extended bioactive peptide sequence containing RGD. One representative example involves the design of a temperature-responsive ELP hydrogel integrated with coiled-coil units (CUBEs). In this system, a bone sialoprotein-derived RGD peptide (bRGD) was incorporated for 3D culture of HUVECs. The results demonstrated that bRGD promoted not only HUVEC adhesion and migration but also angiogenic activity through integrin-mediated interactions (Fig. 6d).^[29]

Beyond the standalone incorporation of the widely utilized RGD peptide, the introduction of various single bioactive peptides into ELP hydrogels can also exert distinct effects on cellular growth and behavior. As an example, the IKVAV adhesive peptide sequence was genetically integrated into an ELP backbone to fabricate a composite hydrogel comprising ELP and functionalized PEG. This system was employed to culture

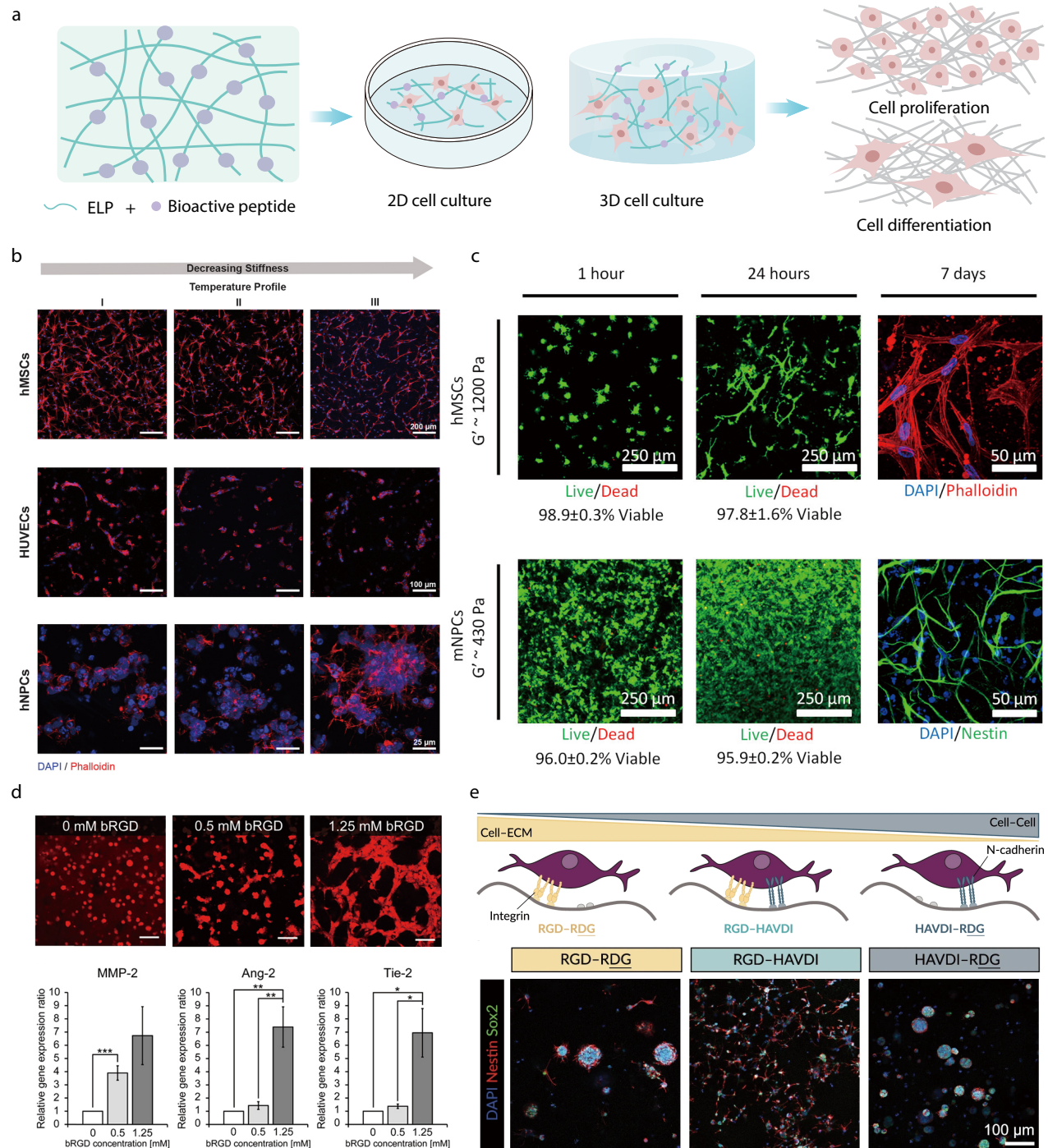


Fig. 6 Functionalization strategies for ELP hydrogels in artificial cell culture. (a) Schematic diagram of 2D or 3D cell culture functionalized with bioactive peptide sequences to guide cell proliferation or differentiation; (b) Cell morphology of hMSCs, HUVECs, and hNPCs cultured for 7 days in HELP-SPAAC hydrogels with stiffness regulated by different temperature profiles. Blue: DAPI-labeled nuclei; Red: phalloidin-labeled F-actin (Reproduced with permission from Ref. [62]; Copyright (2022), Wiley-VCH GmbH). (c) Live/dead staining showed that hMSCs and mNPCs maintained high viability in ELP hydrogels. After 7 days of culture, phalloidin staining revealed the characteristic spread morphology and actin stress fibers (red) of hMSCs, while mNPCs retained the Nestin-positive neural stem cell phenotype (green) (Reproduced with permission from Ref. [45]; Copyright (2017), American Chemical Society). (d) HUVECs were 3D-cultured in bRGD-CUBE hydrogels, and with increasing bRGD concentrations, both the formation of 3D tubular structures and the upregulation of angiogenic markers became more pronounced (Reproduced with permission from Ref. [29]; Copyright (2020), American Chemical Society); (e) Schematic of HELP hydrogels with different ligand combinations to mimic cell-ECM and cell-cell interactions (top). The synergistic effect of RGD and HAVDI allows NPCs to exist in a dispersed network in the rapid stress-relaxation hydrogel, preventing neurospheres formation (bottom) (Reproduced with permission from Ref. [34]; Copyright (2025), The Author(s)).

Table 1 Bioactive peptides used in ELP hydrogels for artificial cell culture.

Category	Bioactive peptide sequence	Origin	Function	Ref.
Cell adhesive peptides	RGD	Fibronectin	Cell adhesion	[29, 34, 35, 45, 61, 62, 65]
	HAVDI	N-cadherin	Cell adhesion	[34]
	IKVAV	Laminin	Cell adhesion	[35, 63, 64, 66]
	YIGSR	Laminin	Cell adhesion	[35]
	DGEA	Collagen	Cell adhesion	[35]
	PLAEIDGIELTY	Tenascin-C	Cell adhesion	[35]
	VFDNFVL	Tenascin-C	Cell adhesion	[35]
Cleavable peptides	GPQGIWGQ	Collagen	Matrix metalloproteinase (MMP) cleavable	[67–72]
	GGGMGPSGPWGK	Collagen	Cathepsin K-sensitive peptide	[70–73]
	GTAR, TSHR, DRIR	Fibronectin	Urokinase plasminogen activator (uPA) cleavable	[74–76]
	GCGPLAQAVRSSRGCG	TNF- α	Disintegrin and metalloprotease 9 (ADAM9) cleavable	[65, 77, 78]
Cell growth factor-derived peptide	KLTWQELYQLKYGI (QK)	VEGF	Promote cell proliferation and angiogenesis	[79, 80]
	NSDSECPLSHDGYCLHDGVC MYIEALDKYACNCVVGIGERC QYRDLKWWELR	HEGF	Promote cell proliferation	[81, 82]
	KIPKASSVPTLSAISTLYL	Bone morphogenetic protein-2 (BMP-2)	Bone regeneration induction	[83, 84]

primary rat sensory neurons isolated from dorsal root ganglia (DRG). Results demonstrated that neurons cultured within IKVAV-presenting hydrogels exhibited a more dispersed distribution and extended longer neurites, indicating pronounced neuroconductive properties of the resulting hydrogel.^[66]

The incorporation of a single bioactive peptide may not always be sufficient to fully support the normal physiological functions of diverse cell types within ELP hydrogels. In view of this, a substantial body of research has further introduced diverse ECM-derived bioactive peptides in synergy with RGD within ELP hydrogel systems, aiming to optimize the cellular microenvironment and expand their applicability in cell research. One representative example is the construction of a modular 3D engineered hydrogel system (HELP) composed of HA and ELP. RGD and HAVDI peptides were incorporated to investigate the maintenance of stemness, long-term expansion, and differentiation of neural progenitor cells (NPCs). The results of combinatorial experiments involving RGD and HAVDI demonstrated that in contrast to either peptide alone, the synergistic effect of RGD and HAVDI enabled NPCs to adopt a dispersed, network-like morphology within viscoelastic hydrogels exhibiting fast stress-relaxing behavior. This distribution pattern supported long-term NPC expansion, preserved stemness, and maintained high cell viability, thereby preventing the formation of necrotic cores typically associated with hypoxia and nutrient deprivation in aggregated neurospheres (Fig. 6e).^[34] The simultaneous incorporation of distinct bioactive peptides alongside the RGD peptide within ELP hydrogels can elicit synergistic effects and further potentiate the bioactivity of RGD. As an illustrative example, a panel of ELP encompassing a range of ECM-derived peptide ligands, including fibronectin (RGD), laminin (IKVAV and YIGSR), collagen (DGEA), and tenascin-C (PLAEIDGIELTY and VFDNFVL), was designed and expressed. The ELP was subsequently reacted with HA to generate a modular and programmable HELP hydrogel system tailored for 3D cell culture. This platform was employed to investigate the outgrowth behavior of embryonic DRG under both mono-ligand and dual-

ligand conditions. The results demonstrated that, among the evaluated ligands, the dual-ligand combination of PLAEIDGIELTY and RGD promoted significantly greater DRG neurite outgrowth than RGD alone at equivalent concentrations.^[35]

Given that a continuous and reciprocal interplay exists between cells and the ECM, cells continuously remodel ECM components in response to their microenvironment. This includes actively updating ECM constituents through mechanisms such as the secretion of degradative enzymes, primarily proteases, to maintain the dynamic equilibrium required for normal cellular function. Although the proteinaceous components inherent to ELP hydrogels are susceptible to degradation by cell-secreted proteases. The degradation kinetics vary considerably depending on the specific protease types, which exhibit differential activity based on peptide sequence and cellular expression profiles.^[85] Notably, intact native elastin is highly resistant to proteolytic cleavage and becomes susceptible only after cumulative damage, such as aging or oxidative stress.^[86] Consequently, the degradation rate of the inherent protein components within ELP hydrogels is relatively slow.^[76]

While this inherent resistance confers long-term structural stability in certain contexts, it limits the ability of cells to remodel their pericellular microenvironment. This remodeling is a critical process for proliferation, migration, and differentiation. To address this, researchers have incorporated within the hydrogel network short peptide substrates susceptible to cleavage by cell-secreted proteases. Upon secretion of the corresponding specific enzymes by encapsulated cells, these peptide motifs are cleaved, leading to localized degradation of the hydrogel matrix. This process creates space for cell proliferation and migration, thereby emulating the dynamic remodeling characteristics of native extracellular matrices. Matrix metalloproteinases (MMPs) are a family of structurally similar zinc-dependent endopeptidases^[67] secreted by cells that degrade a variety of protein components within the ECM, including fibronectin, laminin, collagen, and

gelatin.^[68,69] Leveraging this characteristic of MMPs, numerous studies have incorporated known MMP-specific substrate peptide sequences into ELP hydrogel networks. This strategy enables localized interactions between cells and the hydrogel matrix, allowing the ELP hydrogel to be dynamically remodeled in response to cellular activity. One illustrative example involves the incorporation of specific enzyme-cleavable sites into one ELP sequence, rendering it susceptible to degradation by enzymes that are overexpressed in the post-myocardial infarction microenvironment, including matrix metalloproteinases (MMP-2, MMP-9, and MMP-13) and cathepsin K (Cat K). A second ELP sequence was engineered to contain an RGD motif. Following the modification, these two ELP constructs were assembled into a covalently crosslinked network and injected into the myocardial infarct zone. The results demonstrated that, owing to the degradative activity of both enzyme classes and RGD-mediated cell adhesion, the hydrogel was able to recruit cells and actively create space for their infiltration and migration. This enabled the ELP hydrogel to exert reparative functions at the diseased site requiring intervention, leading to significantly reduced fibrosis, enhanced angiogenesis, and preserved viable cardiomyocytes, thereby achieving improved cardiac function.^[70] Another example also involved the incorporation of cleavage sites for MMP-2, MMP-9, MMP-13, and Cat K into an ELP sequence, which was then used to form a covalently crosslinked network with an RGD-bearing ELP sequence. Three cell types, non-tumorigenic mammary epithelial cells (MCF10A) and two breast cancer cell lines (BCCs), namely MCF7 and MDA-MB-231, were cultured within this system. The results demonstrated that interactions between BCCs and the ECM, including the secretion of ECM proteins and MMPs, contributed to enhanced drug resistance in BCCs, thereby providing a more physiologically relevant model for studying drug resistance and cell invasion.^[71] Using the same sequence design, another study compared the morphology and structure of primary human dermal fibroblasts (HDFa) cultured in hydrogels with or without protease-degradable sequences, as well as the mass loss of the hydrogels during culture. The findings indicated that cell migration and the formation of interconnected networks were achieved through localized degradation and remodeling of the surrounding matrix.^[72]

To enhance the versatility of ELP hydrogels for accommodating a broader spectrum of cell types in culture, it is necessary to incorporate into the hydrogel network specific substrate sequences that can be recognized and cleaved by distinct proteases secreted by different cells. For example, a cleavage site for uPA was integrated into an ELP sequence, which was then crosslinked with PEG to form a uPA-degradable hydrogel. Oligodendrocyte precursor cell (OPC)-like cells were isolated through the Mosaic Analysis with Double Marker technique (MADM OPCs). When cultured within this hydrogel, these cells were shown to express uPA and thereby degrade the hydrogel network, facilitating their transition from an immature state toward maturation.^[75] Another example involved the integration into an ELP sequence of an amino acid motif specifically cleaved by ADAM9, along with the RGD sequence. This construct was crosslinked using tetrakis(hy-

droxymethyl)phosphonium chloride (THPC) to form a hydrogel. Upon culture of NPCs capable of autonomously secreting ADAM9, it was observed that cell-mediated matrix remodeling, achieved through ADAM9 secretion and subsequent degradation of the gel network, activated the transcriptional co-activator Yes-associated protein (YAP) signaling pathway. This activation contributed to maintaining the stemness of NPCs in preparation for subsequent differentiation.^[65]

In the context of artificial cell culture, growth factors are also critically important for regulating cellular behavior. Growth factors can function as soluble signaling molecules that directly determine cell fate *in vitro* and clinical applications. They can also be immobilized or encapsulated within biomaterials or tissue engineering scaffolds to recapitulate the signaling microenvironment of the native ECM. This approach influences cell migration, proliferation, and differentiation in a more sustained and controlled manner. The precise control of this growth factor delivery method, whether using its soluble form or anchoring it, or encapsulating it within a matrix, is one of the core foundations of artificial cell culture for subsequent advanced regenerative medicine research.^[58,76,87] Within ELP hydrogels, one strategy involves anchoring growth factors within the matrix. In one example, a VEGF-mimetic peptide (QK peptide) was covalently immobilized within the gel network. This approach significantly promoted the proliferation of HUVECs in 2D culture. In 3D culture, it facilitated the outward migration and sprouting of HUVEC spheroids, leading to the formation of 3D cellular protrusions.^[80] This matrix-anchoring strategy overcomes the diffusion limitation inherent to small soluble peptides, thereby achieving localized, sustained, and efficient signal presentation. One strategy involves the fusion expression of growth factors with ELP proteins. In one study, epidermal growth factor (EGF) was genetically fused to a human elastin-like polypeptide to generate the HEGF fusion protein, which was subsequently incorporated into a polysaccharide-based 3D printed gel scaffold. Culture experiments using HaCaT keratinocyte cells demonstrated that HEGF embedded within the gel could be released in a bioactive form upon elastase-mediated cleavage, significantly promoting HaCaT cell proliferation.^[82] Another strategy involves sequestering growth factors within the ELP hydrogel network through molecular interactions, thereby achieving sustained effects *via* gradual release. As an illustrative example, a composite hydrogel was constructed based on physical crosslinking of ELP and collagen, within which BMP-2, doxycycline, and 45S5 Bioglass were co-encapsulated. *In vitro* osteogenic differentiation assays demonstrated that the BMP-2-loaded hydrogel significantly enhanced alkaline phosphatase activity and promoted mineral deposition in human adipose-derived stem cells (hASCs). Using a rat calvarial defect model revealed that the hydrogel supported high-quality bone formation and integration. These results indicate that the sustained, slow release of BMP-2 from the ELP hydrogel, in combination with the synergistic interplay among doxycycline and Bioglass, enables efficient and durable induction of bone regeneration.^[84]

In summary, through the leverage of precise sequence programmability and diverse modular design strategies, ELP hydrogels have enabled the construction of an intelligent cell

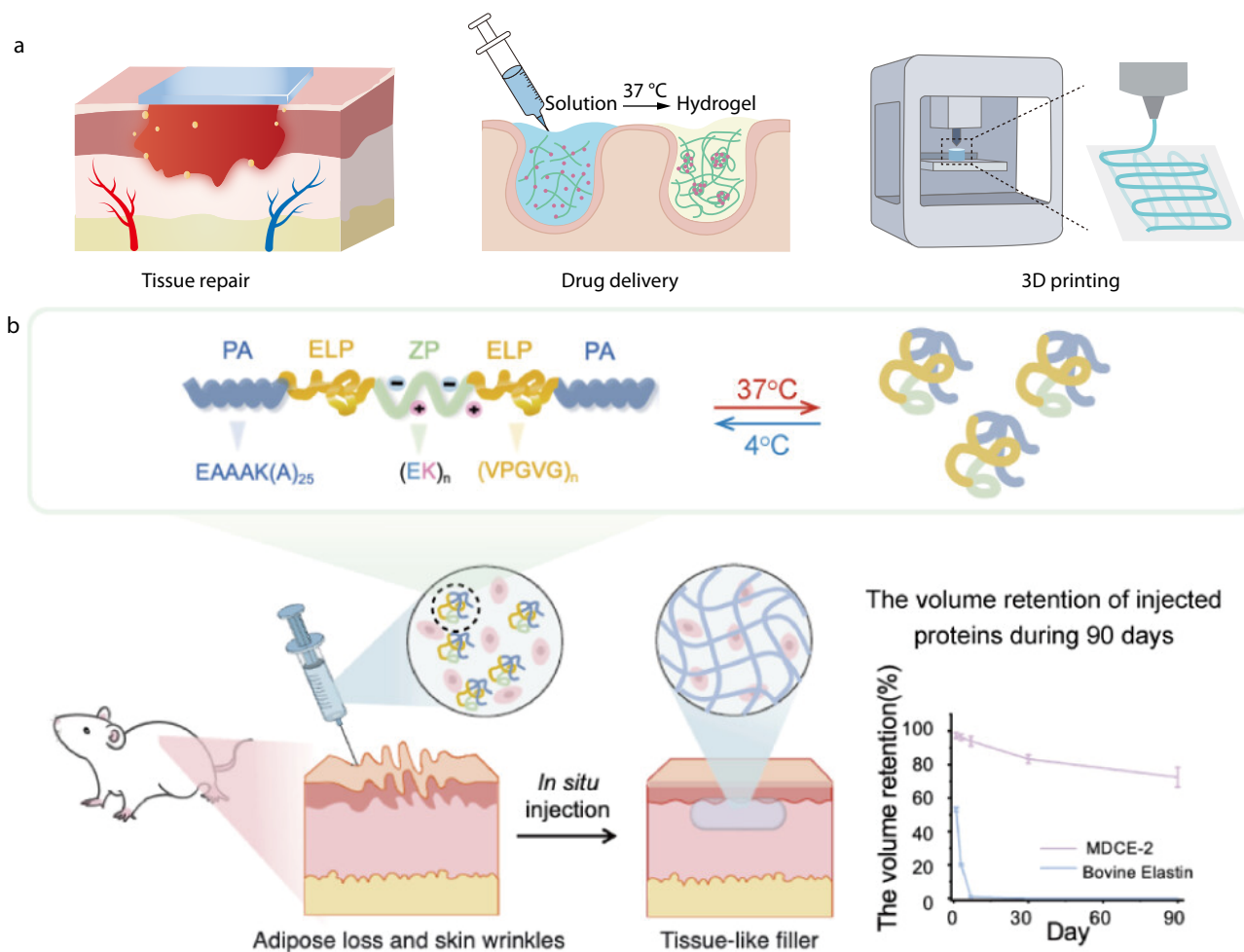
culture platform capable of dynamically responding to and modulating diverse cellular behaviors. This platform not only provides a more biomimetic research tool for elucidating cell-matrix interactions but also establishes a critical foundation for the design and optimization of hydrogel-based cell culture systems toward applications in regenerative medicine.

3.2 Translational Prospects of ELP Hydrogels for Tissue Engineering, Localized Delivery, and Bioprinting

In recent years, researchers have leveraged approaches such as molecular design, optimization of crosslinking strategies, and modular design of functional components. They have developed a range of novel biomimetic hydrogel materials. These materials are characterized by tunable mechanical properties, biological responsiveness to stimuli such as temperature, light, pH, and enzymes, and bioactivity such as antibacterial, antioxidant, and immunomodulatory functions. As a result, their applications in biomedical fields have been substantially expanded. Owing to its thermoresponsive and dynamic crosslinking characteristics, ELP has been widely employed in the development of biomimetic materials for biomedical applications (Fig. 7a). This section is structured into three subsections, covering tissue engineering, localized drug delivery, and bioprinting applications. In the field of tissue engineering, ELP has demonstrated

broad application potential across diverse tissue repair scenarios due to its excellent biocompatibility and thermoresponsive properties.

Cartilage repair provides a representative example. Due to the extremely low cellularity of cartilage tissue, composed mainly of water, fibrillar collagens, non-fibrillar collagens, and negatively charged glycosaminoglycans,^[88] its metabolic activity is low and its self-repair capacity is very limited. Consequently, numerous studies have focused on developing biomaterials for cartilage repair. Leveraging the thermoresponsive properties of ELP, one study constructed a reversibly physically crosslinked ELP hydrogel. Using an ELP sequence with a T_t of 35 °C at a defined concentration, the researchers mixed porcine chondrocytes with the ELP solution and encapsulated them within the gel upon exposure to 37 °C, a temperature above the transition point. The results demonstrated that chondrocytes maintained a well-defined morphology within the ELP hydrogel and synthesized and accumulated sulfated glycosaminoglycans (S-GAG) and collagen comparable to those found in native cartilage tissue, thereby confirming the suitability of the designed ELP hydrogel for *in situ* cartilage repair.^[88] Based on this study, the same material was used to culture human adipose-derived adult stem (hADAS) cells. The results demonstrated that, under hypoxic conditions (5% O₂) and in the absence of TGF- β and dexam-



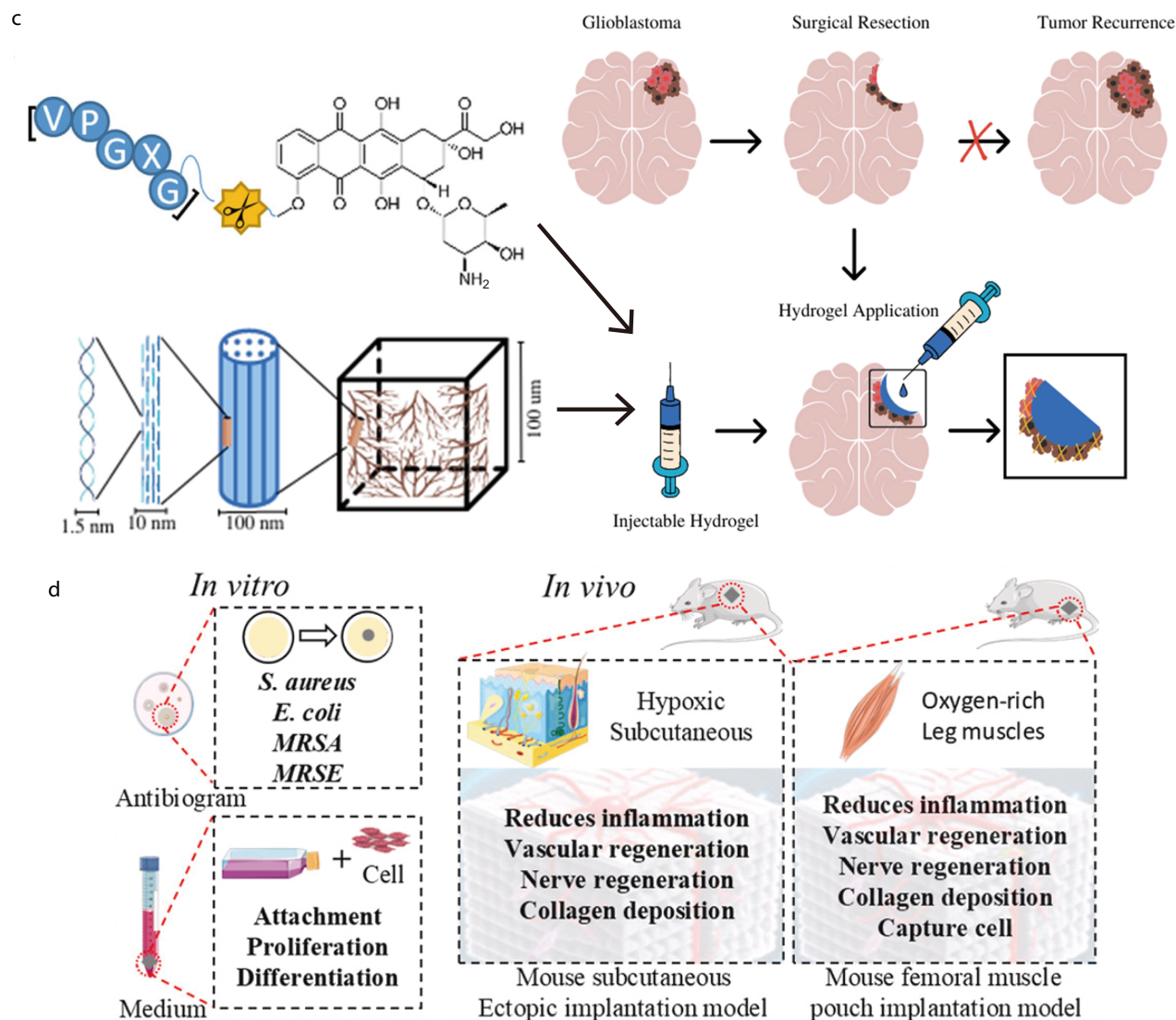


Fig. 7 Temperature-responsive, biocompatible, and sequence-programmable ELP hydrogels for biomedical applications. (a) ELP hydrogel supports tissue repair, drug delivery, and 3D printing; (b) MDCE protein can be injected into the body to form a hydrogel, efficiently filling adipose tissue and maintaining stable volume for 90 days (Reproduced with permission from Ref. [89]; Copyright (2025), American Chemical Society); (c) ELP hydrogel can continuously release drugs locally to inhibit tumor recurrence after brain tumor resection (Reproduced with permission from Ref. [90]; Copyright (2022), The Author(s)); (d) ELP-loaded triclosan-modified PCL scaffolds enhance antibacterial activity, promoting angiogenesis and nerve regeneration (Reproduced with permission from Ref. [91]; Copyright (2025), The Author(s)).

ethasone, the designed ELP hydrogel alone was sufficient to guide hADAS cells toward chondrogenic differentiation, as evidenced by the synthesis of cartilage-specific ECM components, including collagen and sulfated glycosaminoglycans.^[27]

Similarly, peripheral nerve injury (PNI) can result in sensory or motor function never returning to the preinjury level.^[92] Although nerve guidance conduits (NGCs) currently serve as an alternative to nerve autografts, their regenerative outcomes remain limited when used as empty conduits, necessitating the incorporation of a luminal filler.^[93] Owing to its favorable biocompatibility and thermoresponsive properties, ELP is well suited for use as such fillers. One representative example involved the construction of an ELP sequence incorporating the RGD motif, which was covalently crosslinked with THPC to form an initial ELP hydrogel. Owing to the thermoresponsive

properties of ELP, further self-assembly occurred at 37 °C, enhancing the mechanical strength of the hydrogel. In a rat sciatic nerve transection injury model, the repair efficacy of conduits filled with the ELP hydrogel was compared with that of empty conduits. The results demonstrated that the ELP hydrogel, serving as a luminal filler within the empty conduit, significantly improved the rate of nerve bridging and promoted healthy axonal regeneration and remyelination. These findings indicate that the designed ELP hydrogel exhibits tunable mechanical properties, bioactivity, and reproducibility, positioning it as a promising off-the-shelf alternative for promoting nerve regeneration.^[93]

Furthermore, adipose tissue defect repair is a critical clinical requirement in aesthetic medicine and wound healing.^[94] ELP exhibits distinct advantages attributable to its thermoresponsive nature and biocompatibility. One such example is a

fully physically crosslinked, self-assembling multiple-domain-collective elastic-like (MDCE) protein hydrogel, inspired by the synergistic interplay between ordered and disordered domains in elastin and engineered based on the thermoresponsive behavior of ELP. This material was subjected to *in vitro* culture using three distinct cell types and evaluated *via in vivo* injection in a mouse model. The results demonstrated that the hydrogel stably supported essential cellular activities, including adhesion, proliferation, and metabolism, while effectively filling tissue defects *in vivo* (Fig. 7b). Its injectability, tunable modulus, and favorable biocompatibility offer a promising strategy for advancing the clinical translatability of protein-based tissue fillers, particularly for applications in facial rejuvenation.^[89]

In another dimension of soft tissue repair, biomedical adhesives are commonly employed in wound closure, surgical repair, or the reconstruction of degenerated soft tissues.^[95] The thermoresponsive property of ELP can be leveraged to develop such adhesive materials. As an illustrative example, a catechol-modified ELP (Cat-ELP) was engineered, which underwent crosslinking upon exposure to an oxidizing agent to form a hydrogel network. This material was evaluated using adhesion tests on stratified porcine skin. It was also assessed by subcutaneous implantation in mice. The results demonstrated that the Cat-ELP adhesive not only achieved direct and robust bonding to moist tissue surfaces but also exhibited enhanced mechanical stability attributable to the thermoresponsive nature of ELP, while simultaneously displaying favorable biocompatibility and degradability.^[96]

In the context of vascularized tissue construction, vascular endothelial cells (ECs) play a pivotal role in angiogenesis. However, due to their sensitivity to the ECM microenvironment, their physiological activities are tightly regulated by the mechanical properties of the ECM. Native ECM is viscoelastic and exhibits stress relaxation behavior. Nevertheless, conventional materials struggle to achieve precise tuning of stress relaxation kinetics without concomitantly altering other matrix signals, such as stiffness. In contrast, ELP, by virtue of their programmable mechanical characteristics, offer a distinct advantage in the design of materials where stress relaxation can be modulated independently of other biophysical cues. One representative example involved the construction of an RGD-bearing ELP sequence, which was covalently crosslinked with PEG modified with distinct functional groups to form ELP-PEG hydrogels capable of independent modulation of stiffness and stress relaxation kinetics. Angiogenesis assays and *in vivo* subcutaneous implantation experiments were subsequently conducted. The results demonstrated that ELP-PEG hydrogels exhibiting fast stress relaxation and low stiffness optimally supported angiogenesis both *in vitro* and *in vivo*. These findings indicate that the ability to independently tune stress relaxation kinetics in ELP-PEG hydrogels constitutes a critical factor in modulating endothelial cell function, thereby providing a foundational strategy for designing biomaterials that support vascularization within engineered tissues.^[97]

In these diverse repair scenarios, from cartilage and nerves to adipose tissue, wound closure, and vascularization, ELP hydrogels not only demonstrate structural support and tissue

integration capabilities but also actively engage in angiogenesis. This versatility derives from their temperature responsiveness, programmable mechanical properties, and favorable biocompatibility, positioning ELP hydrogels as a versatile tissue-engineering platform and laying the groundwork for broader applications as drug delivery depots and bioink components.

Next, the applications of ELP hydrogels in drug delivery are presented. Drug delivery systems have emerged as a critical research direction in pharmaceutical development and dosage regimen optimization due to their high stability and efficiency.^[98] Hydrogels, as hydrophilic crosslinked networks characterized by a porous architecture, are widely employed in drug delivery applications.^[99] Similarly, benefiting from the programmable thermoresponsive properties and exceptional biocompatibility of ELP, hydrogels constructed from ELP have distinguished themselves among numerous hydrogel materials and have become a research focus in the field of drug delivery. For example, one study covalently conjugated an ELP with the drug molecule doxorubicin (DOXO-EMCH) to form ELP-DOXO. Based on the thermoresponsive properties of ELP, this conjugate transitioned into an injectable, pH-responsive gel state at body temperature (37 °C). Drug release assays and cell culture experiments demonstrated that the designed ELP hydrogel achieved pH-sensitive sustained drug release and was efficiently internalized by tumor cells (Fig. 7c), thereby confirming its significant *in vitro* antitumor activity. These findings provide important support for subsequent preclinical studies in animal models.^[90] One example involved the construction of an ELP sequence incorporating the RGD motif, which underwent gelation at 37 °C based on the thermoresponsive properties of ELP, thereby encapsulating drugs within an injectable ELP hydrogel. Through *in vitro* drug release assays and *in vivo* injection experiments in mice, the ELP hydrogel was demonstrated to exhibit excellent sustained drug release and local drug retention capabilities, significantly enhancing the immunotherapeutic efficacy against melanoma. These findings demonstrate that ELP hydrogels can serve as effective carriers for chemotherapeutic agents and immune checkpoint inhibitors, offering a promising strategy to overcome the current bottlenecks in cancer immunotherapy.^[100] One example involved the construction of ELP-MCC possessing a monofunctional curcumin carbamate (MCC) *via* chemical conjugation. Based on the thermoresponsive properties of ELP, this system underwent gelation at body temperature (37 °C) to form an injectable thermoresponsive drug depot. *In vitro* drug release assays and *in vivo* pharmacokinetic studies in mice demonstrated that the designed ELP hydrogel achieved localized and sustained drug release while reducing systemic drug exposure. This strategy offers an effective approach for treating localized neuroinflammation. Furthermore, the delivery strategy of this carrier can be extended to other polyphenolic drugs, highlighting its adaptability and potential for broader application.^[101]

Finally, the applications of ELP hydrogels in bioprinting are presented. In recent years, various 3D printing technologies have been widely employed in biomedical fields. The development of hydrogel bioinks with excellent rheological properties, rapid gelation capability, and favorable biocompatibility

ty has emerged as a research focus.^[102] In this context, ELP hydrogels have become a class of highly promising biomaterials for bioprinting applications owing to their controllable thermoresponsive gelation, outstanding biocompatibility, and tunable mechanical properties. This promise is exemplified by the integration of a resilin domain, an ELP sequence, an MMP domain, and an RGD domain into a single recombinant protein sequence to serve as a functional bioink. Through mechanical characterization, printability assessment, and cytotoxicity evaluation, the designed bioink demonstrated significantly improved rheological performance, enhanced printing resolution, and superior cytocompatibility.^[103] Another example involved the modification of methionine residues within an ELP sequence with acrylate groups to confer photocrosslinking capability, and GRGDS peptides were grafted onto the ELP chains. Cellulose nanofibers (CNF) were incorporated to modulate the mechanical properties, resulting in a printable bioink exhibiting shear-thinning behavior. This bioink was co-printed with dermal normal human fibroblasts (NHf) and subsequently crosslinked *via* brief UV exposure to generate structurally stable, high-resolution 3D hydrogel scaffolds. The results demonstrated that the ELP hydrogel possessed excellent mechanical properties and favorable biocompatibility, highlighting the potential of ELP-derived bioink for biomedical applications, such as skin reconstruction.^[104] In addition to the direct application of ELP as a bioink for printing, alternative strategies have involved integrating ELP as a key bioactive component into pre-printed 3D gel scaffolds, showcasing the modularity and functionality of ELP and providing important insights for the application of ELP hydrogels in the field of bioprinting. As an illustrative example, owing to its excellent biocompatibility, ELP was grafted onto the surface of 3D printed polycaprolactone (PCL) scaffolds *via* a conjugation reaction. This modification substantially enhanced the biocompatibility and cell affinity of the PCL scaffolds, promoting angiogenesis and nerve regeneration. Concurrently, the ELP component served as a carrier enabling the controlled release of the antimicrobial agent triclosan, achieving effective inhibition of drug-resistant bacteria (such as methicillin-resistant *Staphylococcus aureus*) while mitigating drug toxicity (Fig. 7d). These findings highlight the considerable promise of ELP in the development of printable biomaterials for tissue regeneration applications.^[91]

In summary, these application scenarios highlight a core advantage of ELP hydrogels: a genetically programmable and modularly designed material platform that can be broadly adapted to diverse biomedical contexts by modulating sequence design and crosslinking logic. This platform significantly reduces translational barriers and offers new strategies for rational optimization toward clinical and bioengineering applications.

4 CONCLUSIONS AND OUTLOOK

We have reviewed the research progress of ELP hydrogels from the perspectives of crosslinking modalities (network types), cell culture (property regulation), and biomedical applications (functionalization).

With regard to crosslinking strategies, tailored mechanical

properties can be achieved through precise genetic encoding of ELP sequences and targeted chemical modification of ELP polypeptide chains, combined with diverse physical and covalent crosslinking approaches including thermoresponsive assembly, photocrosslinking, and enzymatic crosslinking. Turning to their applications, *in vitro* studies have demonstrated that ELP hydrogels, through the incorporation of bioactive peptides containing cell-adhesive, degradable, or pro-growth factors, achieve biomimetic microenvironments and enable interactions such as guiding cellular behaviors. *In vivo* applications have confirmed that ELP hydrogels meet the diverse requirements of tissue repair, drug delivery, and bioprinting, demonstrating their efficacy, biocompatibility, and functionality.

Taken together, based on their genetically programmable sequence, excellent biocompatibility, and tunable thermoresponsive properties, ELP hydrogels have emerged as a highly promising platform in the biomaterials field, spanning from fundamental research to clinical translation. Looking forward, numerous opportunities for further investigation remain in the study of ELP hydrogels, ranging from molecular-level material design to functional applications and ultimately to clinical implementation.

In terms of material design, future efforts can focus on extending the controllable advantages inherent to ELP hydrogels toward two key directions: dynamic and intelligent responsiveness, and multi-domain integration. Regarding dynamic and intelligent responsiveness, dynamically adaptive ELP hydrogels endowed with self-healing, stress relaxation, and reversible remodeling capabilities can be developed to better mimic the dynamic ECM that supports cell growth and living tissues.^[105] For instance, this can be achieved by introducing dynamic covalent bonds^[106] or supramolecular interactions, such as peptide self-assembly.^[107] One example involves the introduction of dynamic non-covalent interactions through sequence engineering, incorporating pH-sensitive charged residues (glutamic acid and histidine) along with hydrophobic groups into the ELP sequence. This design enables the physical interactions (electrostatic and hydrophobic) between polypeptide chains to respond sensitively and reversibly to environmental pH, thereby triggering rapid liquid-liquid phase separation in solution and forming discrete liquid condensates.^[108] This integration of environmental sensitivity with intrinsic phase-separation propensity offers a conceptual reference for the future development of smart, dynamically responsive ELP hydrogel systems capable of feedback-driven sensing and response, enabling signal-dependent gel functional switching and matrix remodeling.

More significantly, the self-healing advantages of future dynamically responsive and intelligent ELP hydrogel scaffolds will no longer be confined to conventional physical or covalent crosslinking reconstruction. Instead, by integrating synthetic biology with material design^[109], researchers can construct living ELP hydrogel systems based on engineered bacteria. Through genetic engineering of bacterial strains (such as *Escherichia coli*, *Bacillus subtilis*, and *Lactococcus lactis*), these microorganisms can be incorporated as active and intelligent components within the hydrogel.^[110–115] This endows ELP hydrogels with damage-responsive modules (such

as pH responsiveness^[116], light responsiveness^[117]) and continuous protein synthesis modules (encoding recombinant ELP, among others).^[118] Within this design framework, ELP hydrogels are no longer merely passive structural scaffolds but active participants in the living material system. They can serve as a programmable structural matrix for bacterial colonization, supplying a 3D scaffold for the encapsulated engineered bacteria. They can also function as bacterial secretion products, enabling dynamic renewal of the hydrogel matrix. By leveraging gene editing to integrate bacterial sensing and synthetic capabilities within the ELP hydrogel, this system effectively bridges living cells with synthetic materials. This confers upon ELP hydrogels the capacity to adapt their composition and properties in response to environmental signals, thereby enabling the development of self-growing and self-healing living ELP hydrogel materials. Regarding multi-domain integration, current research on ELP hydrogels remains largely at the stage of simple fusion of one or a few functional domains. In the future, this should evolve toward a modularly designed, programmable intelligent biomaterial platform that integrates multiple functions in a unified system.^[119] The core design concept lies in leveraging genetic engineering to incorporate a series of peptide segments encoding specific functions as independent modules into the repetitive ELP sequence, thereby achieving precise editing of the functional modules within ELP hydrogels. As discussed above, numerous studies have already incorporated bioactive domains into ELP hydrogels. Beyond this, future iterations could integrate additional functional elements such as self-assembling peptides, antimicrobial peptides, enzyme-cleavable peptides, and short peptides derived from functional proteins (such as specific active fragments from native mineralization-related proteins).^[58] Such an approach would enable a single ELP-based material to simultaneously integrate multiple capabilities, including cell adhesion, environmental responsiveness, on-demand remodeling, modulation of biochemical signals, and tunable mechanical properties. This marks a paradigm shift for ELP hydrogels, transitioning from passively adaptable gel scaffolds toward biomimetic, dynamically interactive platforms capable of actively participating in and directing complex biological activities.

In terms of functional applications, although current ELP hydrogels are suitable for cell culture and widely used in conventional tissue engineering, drug delivery, and bioprinting, the hydrogel scaffold itself remains at a stage of passively providing space for growth and is unable to accurately replicate the highly dynamic and complex cellular microenvironment found *in vivo*. Consequently, they still face fundamental challenges in application areas that demand highly specific biological stimulation signals. Future breakthroughs in the functional applications of ELP hydrogels should be grounded in the innovative integration of materials design and advanced technologies, with a focus on cutting-edge fields requiring high precision and dynamic biomimicry, such as microfluidics^[120–125] and organoid^[126–131] construction. Leveraging the thermoresponsive and genetically programmable advantages of ELP, they can be developed as the primary material for microfluidic chip fabrication. Through bioprinting techniques, microchannel networks with tailored stiffness

gradients, adjustable densities of cell-adhesive ligands, and sustained release of growth factors can be constructed. For example, cells encapsulated within such systems could spontaneously assemble to form vascular networks or, under external induction by growth factors, undergo adipogenesis to generate adipose tissue.^[132] Furthermore, utilizing the thermoresponsive properties and excellent biocompatibility of ELP, they can serve as matrices for 3D organoid culture.^[133,134] Building upon this foundation, dynamically responsive ELP hydrogels can be developed, integrating temporal control over the release of growth factors that activate organoid differentiation pathways, as well as modulating local gel degradation rates and mechanical properties. This approach would enable the creation of a 4D culture matrix capable of precisely recapitulating the signaling stimuli and matrix remodeling dynamics that occur during organoid development.^[135] Furthermore, ELP hydrogel scaffolds can be integrated with microfluidic technology to construct organoid-on-a-chip platforms for differentiation. Such platforms can be utilized for the fabrication of vascularized organoids, offering new methodologies for high-throughput drug screening and the establishment of personalized disease models. This approach holds promise for creating a next-generation biomedical research platform capable of dynamically simulating complex organ development, growth, and disease processes, thereby advancing the field of biomedical and tissue engineering to a higher dimension.^[136–138]

With the continuous advancement of the field of artificial intelligence (AI), protein research is no longer solely reliant on traditional experimental approaches. An increasing number of protein sequence designs, structures, and other attributes are being validated through AI models.^[139–147] For instance, one study employed an AI-driven computational model to design a novel sequence (AI-PH_n) capable of adopting an α -helical conformation, which was subsequently integrated into an ELP sequence to generate an ELP variant with tunable phase behavior and self-assembly properties.^[148] In the future, the synergistic integration of AI and big data era is expected to further expand protein databases^[149] and yield an increasing number of *de novo* designed sequences, thereby enabling the development of hydrogels with an even broader range of tunable functionalities.^[150] For ELP hydrogels, current sequence design mostly relies on experimental validation. Consequently, the relationships among complex sequences, structures, properties, and functions are difficult to systematically analyze. This is a significant challenge for their application in precision medicine. In the future, a multidimensional database could be established. This database would integrate ELP sequence information, hydrogel mechanical properties (such as crosslinking methods, elastic modulus, and swelling ratio), cellular responses (including survival, proliferation, and differentiation), and functional applications (such as bioprinting and tissue repair). With such a database, forward prediction from sequence design to macroscopic performance could be achieved. On this basis, a reverse strategy could be further introduced. This strategy would start from target mechanical properties or desired cellular behaviors and combine machine learning models to screen optimal ELP sequences. These two predictive approaches would

transform ELP hydrogel development from a purely experimental-validation model to a combination of theoretical modeling and experimental validation. This would significantly shorten the development cycle for obtaining the optimal ELP hydrogel for specific applications. It would also promote the efficient design of multifunctional smart biomaterials. Ultimately, it would achieve the goal of providing customized and diverse solutions for clinical needs.^[151,152]

In summary, ELP hydrogels represent a pioneering and highly promising class of biomaterial platforms. They are distinguished by their sequence-level programmability, thermoresponsive assembly behavior, and modular functionality. Continued advances in molecular design, predictive modeling, and system integration technologies are expected to transform ELP hydrogels from adaptable scaffold materials into clinically applicable, smart responsive materials capable of actively directing complex biological processes.

BIOGRAPHIES

Bin Xue has been an associate professor at the School of Physics, Nanjing University, since 2023. He received his Ph.D. in Physics from Nanjing University in 2017. His research focuses on the physical and mechanical design and regulation of bio-based soft materials. Centering on the multiscale mechanics of soft matter, he constructs hierarchical structures that couple biomolecular assemblies with mechanically controllable soft carriers, and systematically studies their mechanical mechanisms and applications in areas such as cell mechanics, tissue engineering, and flexible sensing.

Yi Cao has been a professor at Nanjing University since 2010. He received his B.Sc. in Polymer Chemistry from Nanjing University in 2001, where he also earned his M.Sc. in Polymer Chemistry and Physics in 2004. He received his Ph.D. in Chemistry from the University of British Columbia in 2009. His research focuses on better understanding the design principles of natural materials with excellent mechanical properties, as well as those of engineered synthetic soft materials with similar or even superior mechanical properties. By combining single-molecule force spectroscopy with synthetic biology and polymer chemistry, he addresses fundamental problems and application challenges in this interdisciplinary field.

Conflict of Interests

The authors declare no interest conflict.

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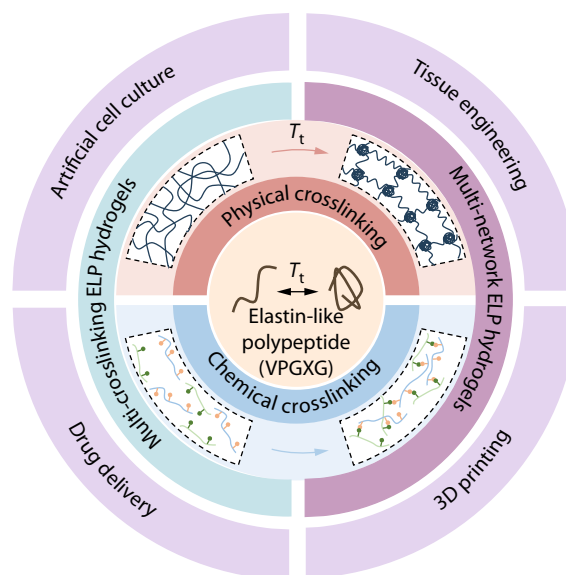
Graphical Abstract

Engineering Elastin-like Polypeptide Hydrogels: from Design Strategies to Cellular and Biomedical Applications

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This review summarizes elastin-like polypeptide (ELP) hydrogel design and functionalization strategies and highlights their applications in cell culture and other biomedical fields. Finally, it envisions dynamic, smart, integrated ELP-based biomaterial platforms.



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